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Naphthenic acids in oil sands process waters: Identification by conversion of the acids or esters to hydrocarbons

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Abstract

Concerns over the toxicity associated with 'naphthenic' acids (NA) within oil produced waters and oil sands process waters (OSPW), whether justified or not, have increased the need for the structural elucidation of NA. Certainly, oil sands mining operations, such as those in Alberta, Canada, result in the production of large volumes of OSPW contaminated with NA. Monitoring NA, predicting the toxicities and accounting for the toxicity of residual NA after clean-up treatments, have all been hindered by the lack of NA identifications. Recently, a method involving the conversion of the esters of petroleum NA to the corresponding hydrocarbons before analysis by comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC-MS), resulted in the most comprehensive study of bicyclic NA from petroleum to date. Here, we present results of the analysis by GC×GC-MS of NA extracted from OSPW after conversion of the acids or ester derivatives to the corresponding hydrocarbons. The identifications presented include novel alicyclic, aromatic and sulphur-containing hydrocarbons and thus, by inference, of the corresponding acids. This supports and significantly extends, previous identifications of OSPW NA as their methyl esters and can now be used to better inform environmental monitoring programs and toxicity studies.

Keywords

naphthenic acids, GCxGC-TOF-MS, oil sands process water, OSPW

1. Introduction

The identification of individual petroleum or 'naphthenic' acids (NA) has proved challenging for analytical and environmental geochemists for over a century (e.g. Hell and Medinger (1874)), owing to the complexity and polarity of the isolated acid mixtures. Early research focused on the role of NA as geochemical markers, their interfacial and corrosive properties and industrial uses of their metal salts (Seifert, 1975). However, the structural identification and monitoring of NA has gained considerable interest in recent years, due to concerns over the presence of NA in oil produced waters and oil sands process waters (OSPW) and their associated toxicities (Thomas et al., 2009), particularly at the concentrations reported in the OSPW storage lagoons (e.g. reviewed by Jones et al. (2011); Yue et al. (2015a) and references therein).

Thus, intensive surface mining of bituminous oil sands, such as those of Alberta, Canada, produces large volumes of OSPW. Despite recycling of the caustic extraction water, large volumes of process water, contaminated with water-soluble organics from the bitumen, such as NA, as well as suspended solids, continue to accumulate and are stored in tailings ponds and lagoons (Kean, 2009).

Undiluted OSPW have been shown to be toxic to numerous organisms at the concentrations found in tailings ponds and since this toxicity has been largely attributed to the NA content (He et al., 2012; Marentette et al., 2015), considerable effort has been made to develop methods for monitoring the extremely complex distributions of NA found in OSPW (Frank et al., 2016). High and ultra-high resolution mass spectrometry methods have revealed that OSPW contain an extensive range of NA (Yi et al., 2015), beyond so-called 'naphthenic' or cyclic non-aromatic species (Rowland et al., 2014; Barrow et al., 2015). Many analytical methods also include a chromatographic separation step (e.g. GC or UPLC) (Ortiz et al.,

2014; Brunswick et al., 2016; Headley et al., 2016), but most still lack sufficient separation for identification of individual NA in the OSPW mixtures.

However, the use of comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC–MS) for the analysis of NA as methyl esters has led to much better separations of NA mixtures in OSPW. Thus, several bicyclic, tri- and pentacyclic diamondoid acids and diacids have been identified by comparison of GC×GC retention times and mass spectra with those of authentic compounds (Rowland et al., 2011b; Rowland et al., 2011d; Lengger et al., 2013). However, the number of identifications possible was still limited by the complexity of the acid mixtures, the sparsity of data in current mass spectral databases for relevant esters and the poor availability of reference compounds for confirmation (Wilde et al., 2015).

Recently, a complementary method for the identification of NA was reported in which, after calibration of the method with model acids, NA were first converted to the corresponding hydrocarbons (Wilde and Rowland, 2015). This approach, based on refinements of chemical transformation methods used in early investigations of NA (Seifert et al., 1969), coupled with analysis of the reduced acids (viz: hydrocarbons) by GC×GC-MS, resulted in the identification of over 40 individual bicyclic acids in a commercial NA mixture isolated from petroleum. Identification of the bicyclanes and thus, by inference, of the original bicyclic acids, was achieved by mass spectral comparison: many more published mass spectra exist for reference hydrocarbons than for acids or esters (Denisov et al., 1977a; Denisov et al., 1977b; Denisov et al., 1977c; Golovkina et al., 1984; Petrov, 1987). This made comparisons of reference mass spectra with those of the unknowns, much more feasible than for the esters.

Following the success of the conversion approach on petroleum NA, the current investigation aimed to apply the same method to the even more complex NA extracts of some OSPW.

2. Methods

Two NA extracts from OSPW tailings ponds from Alberta, Canada, were analysed. Sample #1 was an NA extract from OSPW collected from the West In-Pit (WIP) tailings pond in 2009, and prepared as described previously (Frank et al., 2006). Briefly, a cleaned-up concentrated naphthenate solution was obtained by passing the re-dissolved precipitated acids from OSPW through a diethylaminoethyl-cellulose column (Frank et al., 2006). Sample #2 was OSPW from a second tailings pond. This OSPW was filtered, acidified and extracted in DCM to provide a crude NA extract (cf. Pereira et al. (2013)).

2.1. Instrumentation

Comprehensive GC×GC-MS analyses were conducted on an Agilent 7890A gas chromatograph (Agilent Technologies, Wilmington, DE) fitted with a Zoex ZX2 two stage cooled loop GC×GC modulator and secondary oven (Houston, TX, USA) interfaced with a BenchTOFdx™ time-of-flight mass spectrometer with an electron impact ionisation source (Markes International, Llantrisant, Wales, UK). The primary column was a 60 m × 0.25 mm × 0.25 μm Rxi®-1ms (Restek, Bellefonte, USA), followed by a 1 m x 0.1 mm deactivated fused silica modulation loop. The secondary column was a 2.5 m × 0.1 mm × 0.1 μm BPX50 (SGE, Melbourne, Australia). Helium was used as carrier gas and the flow was kept constant at 1.0 mL/min. 1 μL samples were injected at 275 °C splitless. Samples were analysed using two temperature programmes (referred to as condition sets A and B). Condition set A was described previously (Wilde and Rowland, 2015) and are given in the Supporting Information. Condition set B involved the primary oven programmed from 40 °C, held for 1.3 min, then heated to 200 °C at 1 °C/min, to 280 °C at 5 °C/min and to 320 °C at 10 °C/min and then held for 5 min. The secondary oven was programmed to track the primary oven at 20 °C above. The hot jet was programmed to start 20 °C above the primary oven and finish 100 °C above the primary oven over the period of the run; programmed from 60 °C, held for

1 min, then heated to 200 °C at 1.1 °C/min, to 280 °C at 3 °C/min and then to 400 °C at 5 °C/min. Modulation periods of 4 and 6 s were used. The MS transfer line and ion source temperature were 290 °C and 300 °C, respectively.

Data processing was conducted using GC Image™ v2.3 (Zoex, Houston, TX, USA). The CLIC (Computer Language for Identifying Chemicals) expression tool within GC Image™ v2.3 was used for the determination of the presence or absence of compounds. CLIC expressions are a powerful tool for applying mass spectral filters and produce advanced extracted ion chromatograms (EICs) with additional constraints (e.g. Figure 1) (Reichenbach et al., 2005). Some mass spectral matches were made using NIST Search MS 2.0. Reference mass spectra obtained from the literature were input into a local NIST library using the NIST librarian tool to allow mass spectral match quality values to be determined; typically >85 %.

2.2. Derivatisation and Fractionation of NA (Scheme 1)

Sample #1 was first derivatised by heating with BF_3-methanol (BF_3-MeOH) and the esters fractionated by argentation (Ag-Ion) chromatography using a method described previously (Scarlett et al., 2013) and outlined in the Supporting Information. Three fractions were used for conversion to hydrocarbons; an 'alicyclic' fraction (F2) eluting with 100% hexane; an 'aromatic' fraction (F5) eluting with 95 %:5 % hexane:ether and an 'aromatic/sulphur'-containing fraction (F7) eluting later with 95 %:5 % hexane:ether. The NA extract of Sample #2 was not derivatised or fractionated prior to the conversion to the hydrocarbons: i.e. the free acids were reduced to the hydrocarbons, avoiding the esterification step (Scheme 1).

2.3. Conversion of Acids and Esters to Hydrocarbons

Sample #1 (methyl esters) and sample #2 (free acids) were converted to hydrocarbons via the three-step transformation developed and described previously (Scheme 1) (Wilde and Rowland, 2015). Briefly, the acids and ester derivatives were reduced using lithium

aluminium hydride (LAH) to the primary alcohols. The alcohol products were derivatised to the p-toluenesulfonate esters using tosyl chloride (TsCl) in the presence of 4-(dimethylamino)pyridine (DMAP) and triethylamine (TEA). The tosylate products underwent a 'SuperHydride®' reduction with excess lithium triethylborohydride (LiEt3BH) to produce the hydrocarbons. To retain any volatile, low molecular weight products from conversion of sample #2 (shown by preliminary GC-MS to contain more abundant low molecular weight, volatile esters), the sample was concentrated to ~1.0 mL using a Kuderna-Danish apparatus, followed by a simple silica chromatography clean-up step (Wilde and Rowland, 2015). The masses obtained from the fractionation procedures and the yields of the conversion reactions are detailed in Tables S1 – S3 in the Supporting Information.

3. Results and Discussion

Fractionation of the NA extract of sample #1 by Ag-Ion chromatography (as methyl esters; Scheme 1), produced 'alicyclic' (36 %), 'aromatic' (17 %) and 'aromatic/sulphur' (4 %) sub-fractions of OSPW. These contrasted with the proportions of sub-fractions of a commercial NA extract obtained from the refinement of petroleum (80.0 %, 6.4 % and 0.9 % respectively) (Wilde and Rowland, 2015) but were consistent with the results of previous fractionation studies of OSPW NA analysed by liquid chromatography with ion mobility and high resolution mass spectrometry (Huang et al., 2016).

3.1. Identification of NA after reduction to hydrocarbons

3.1.1. Adamantane Acids

Previously, tricyclic 'diamondoid' or adamantane acids, including diacids, have been identified in OSPW and oil sands composite tailings pore water, as their methyl ester derivatives using GC×GC-MS (Rowland et al., 2011b; Bowman et al., 2014; Lengger et al., 2015). Therefore there is precedent for the identification of adamantane acids within some

OSPW NA. Thus, if the conversion of the more complex OSPW NA (free acids or esterified acids) herein had been successful, analysis of the reduced acid products should lead to the identification of the corresponding alkyl adamantane hydrocarbons.

The mass spectra of alkyl adamantanes are very distinctive and many reference mass spectra are available in the literature: diamondoid hydrocarbons are commonly used as biomarkers in petroleum geochemistry (Peters et al., 2005). The spectra of alkyl adamantanes usually display a dominant or base peak ion corresponding to the loss of the largest alkyl group as a radical, with the molecular ion for isomers substituted at tertiary carbons displaying a relatively low intensity molecular ion (approx. 5 – 20 %), compared with isomers substituted at secondary carbon positions (approx. 30 – 50 %) (Golovkina et al., 1984). The GC elution order of many dimethyl-, ethyl-, methylethyl- and trimethyl adamantane isomers is documented (Wingert, 1992; Wei et al., 2006; Wang et al., 2013): typically, alkyl adamantanes substituted at the secondary carbon positions elute later in GC (and GC×GC) than isomers substituted at the tertiary positions (Petrov, 1987).

The high complexity of the NA extracts from OSPW has hindered previous identifications and even extracted ion monitoring of the GC×GC data can still result in very complex chromatograms. However, use of CLIC expressions (cf. Wilde and Rowland (2015)), derived from the key mass spectral features for alkyl adamantanes, simplified the resulting chromatograms, displaying more clearly a series of isomers identified as mono- and poly-substituted alkyl adamantanes by comparison with reference mass spectral data and known elution orders (Figures 1, S1 and S2).

Assignment of the two ethyl isomers of adamantane in the reduced sample #2, after comparison with the spectra reported by Polyakova et al. (1973) (Ad-II and V; Figure 1; C-F) also allowed identification of numerous dimethyl- isomers (Figures S1 and S2) based on the

comparison of the retention positions, relative to the ethyl isomers. For example, the only two dimethyladamantane isomers reported to elute between the two ethyl isomers are the cis- and trans- isomers of 2,4-dimethyladamantane (Wei et al., 2006; Wang et al., 2013). In the present study this assignment was supported by the high intensity molecular ions (50 – 60 %) observed in the mass spectra of peaks Ad-III and –IV (Figure S1).

The earliest eluting peak (Ad-I) was assigned as 1,2-dimethyladamantane. The absence of 1,3-dimethyladamantane, expected to elute before the 1,2-dimethyl- isomer was consistent with the absence of 3-methyladamantane-1-carboxylic acid methyl ester, after comparison of the retention time and mass spectrum of the reference acid methyl ester with the original NA methyl esters. The adamantane ethanoic acids were also identified in the methyl esters of sample #2 by comparison of data with those for reference compounds; once more indicating the complementary nature of the analysis of both the esters and hydrocarbons by GC×GC-MS. The identification of these acids as the hydrocarbons in the reduced non-methylated, unfractionated OSPW free NA (#2), also showed that the acids are not artefacts of esterification.

The reduced samples #1 (reduced methyl esters of NA) and #2 (reduced, unesterified free NA) also showed series of peaks displaying molecular ions at *m/z* 178, corresponding to C_{13} tricyclic hydrocarbons, many of which had mass spectra matching the reference spectra of trimethyl- and methylethyl- adamantanes (Figures 1 and S2). The additional clarification of peaks provided by the CLIC expression chromatogram, allowed comparison of the retention positions of the C_{13} adamantane isomers with those reported in the literature to aid identification (Figure 1; A and B).

These results confirmed that the conversion of OSPW NA mixtures to the corresponding hydrocarbons was successful, despite the increased complexity compared to the petroleum

209 acid mixtures analysed previously (Wilde and Rowland, 2015). Thus, due to the widely
210 available reference spectra and the known elution orders of numerous alkyl adamantanes,
211 conversion of the OSPW NA as acids or esters, to the corresponding hydrocarbons,
212 confirmed the presence of known acids, the presence of acids that were only tentatively
213 assigned previously and also resulted in the identification of numerous new acids.

214 **3.1.2. Bicyclic Acids**

215 Alicyclic bicyclic acids are a major class of OSPW NA. High- and ultra-high resolution mass
216 spectrometric techniques often show ions corresponding to $C_nH_{2n-4}O_2$ acid species with
217 carbon numbers (n) ranging from C_{9-20}, as the most abundant $C_nH_{2n+z}O_2$ species (z, hydrogen
218 deficiency due to cyclicity) (Barrow et al., 2010). Analysis of commercial and OSPW NA
219 methyl esters by GC×GC-MS has shown that the ions detected by high resolution mass
220 spectrometry alone represent hundreds of different structural isomers (Damasceno et al.,
221 2014; Wilde et al., 2015). Based on the limited knowledge of bicyclic acids, a well-accepted
222 screening assay reported some bicyclic acids to be the most toxic of those studied (Jones et
223 al., 2011; Yue et al., 2015b). Scott et al. (2008) showed the ozonation of OSPW resulted in
224 the removal of most bicyclic acids ($C_nH_{2n-4}O_2$ species); however, little is known about the
225 residual acids, or of any transformation products.

226 Investigations into the structural identification of bicyclic acids resulted in the first
227 identification of novel bridged bicyclic acids, along with several bicyclic fused and
228 terpenoid-derived acids (Wilde and Rowland, 2015; Wilde et al., 2015). In the current
229 investigation, analysis of the 'alicyclic' acids as methyl esters of sample #1 and the
230 unfractionated acid methyl esters of sample #2, revealed homologous series of isomers with
231 mass spectra displaying molecular ions consistent with that of C_{11-16} and C_{10-15} bicyclic acids
232 respectively, as reported as typical of OSPW NA (Hindle et al., 2013). Careful concentration
233 of the hydrocarbon product of sample #2, following reduction of the unfractionated NA,

using a Kuderna-Danish apparatus and subsequent analysis by GC×GC-MS showed homologous series of C_{10-15} bicyclanes (Figure S3; A and B).

Despite the complexity of sample #2, comparison of the mass spectra of those bicyclanes which were not hindered by co-elution effects, resulted in the identification of bicyclo[3.2.1]octane, bicyclo[3.3.0]octane and bicyclo[4.3.0]nonane alkyl derivatives (Figure 2 and S3). For example, the earliest eluting isomer (**bi-I**) was assigned as 1,4-dimethylbicyclo[3.2.1]octane (Figure 2; A and B) after comparison with reference mass spectra (Denisov et al., 1977a). The same bicyclane, and by inference the same acid, was identified previously in petroleum-derived NA (Wilde and Rowland, 2015).

Comparison of the mass spectrum of isomer bi-II with the mass spectra of alkyl bicyclo[3.3.0]octanes resulted in the identification of 1,2-dimethylbicyclo[3.3.0]octane (Figure 2; C and D) (Denisov et al., 1977c). This assignment was supported by its retention position relative to 1,4-dimethylbicyclo[3.2.1]octane (bi-I) which matched the elution order of C_{10} bicyclanes reported previously (Piccolo et al., 2010) and complemented the identification of several bicyclo[3.3.0]octane acids in petroleum-derived NA (Wilde and Rowland, 2015). However, this was the first identification of a bicyclo[3.3.0]octane acid in NA with a substituent at a bridgehead carbon.

The isomer bi-III displayed a mass spectrum matching those of *cis-cis* and *trans-cis* 3-methylbicyclo[4.3.0]nonane (Figure 2; E and F) (Denisov et al., 1977b). Numerous methylbicyclo[4.3.0]nonanes were identified in the petroleum NA (Wilde and Rowland, 2015) with the isomer assigned as the *cis-cis*/*trans-cis* 3-methyl- isomer eluting earliest of all the 2- and 3-methyl- isomers. The mass spectra of the later eluting isomers bi-IV to VI were difficult to assign. The isomer bi-IV was tentatively assigned as 2,6-dimethylbicyclo[3.2.1]octane (Figure S3; C and D) and the mass spectra of the remaining

bicyclanes, e.g. bi-V and VI (Figure S3; E and F), did not match any of the reference spectra, which encompassed an extensive range of bicyclane isomers.

Petroleum NA and NA extracted from OSPW have often been reported to possess significantly different compositions (Grewer et al., 2010). This is often observed as differences in the HPLC retention times of the unresolved NA 'humps' (OSPW NA eluting slightly earlier) (Han et al., 2008). The differences are also reflected in the increased resistance of OSPW NA to biodegradation; suggested to be because the OSPW NA possess more highly branched carboxylated side chains (Misiti et al., 2014; Brown and Ulrich, 2015). However, this speculation has never been supported by the identification of the acids. The presence of aromatics in OSPW NA, may have an important role in the differences in retention time and toxicity observed for different NA mixtures (Jones et al., 2012), but aromaticity obviously does not explain the observations made for these alicyclic acids.

A complementary explanation suggested here is that the OSPW NA examined herein instead possess a higher proportion of condensed, bridged structures, substituted at different positions e.g. bridgehead carbon (Figure 2; A-D). Differences between the bicyclic acids, identified as the corresponding bicyclanes, in petroleum NA and OSPW NA included the position of substituents (e.g. bridgehead substituted isomers present in OSPW, Figure 2; C), as well as the presence of some unknown bicyclic acid isomers detected in OSPW, but not detected in petroleum NA (e.g. Figure S3). The suggestion is supported by the fact that bridged compounds elute earlier than fused ring acids (e.g. decalin acids) and is reinforced by the identification of bicyclo[2.2.1]heptane acids in OSPW and the reported elution order of reference acids (Wilde et al., 2015). Bicyclo[2.2.1]heptane acids are the earliest eluting of any the bicyclic acids previously analysed, which were not detected in petroleum NA as the methyl esters, or corresponding bicyclanes (Wilde and Rowland, 2015; Wilde et al., 2015).

These structural differences may also help explain the differences in toxicity of some NA mixtures.

Despite recent advances in spectrometric studies on OSPW and NA (Headley et al., 2016), few examples exist which have provided structural evidence for the observed compositional differences between different NA mixtures based on the identification of individual acids (Swigert et al., 2015). Following the identifications herein; representative NA can now be used to quantify the relative proportions of bicyclic species by high resolution methods; relevant acids can be synthesised and the toxicities measured (Yue et al., 2015b).

3.1.3. Non-Diamondoid Tricyclic Acids

Apart from the adamantane acids, few tricyclic acids have been identified in OSPW to date. The mass spectra of isomers tt-I to -VI all displayed fragmentation patterns characteristic of those of tricyclic terpane hydrocarbons (Figure S4). Ekweozor and Strausz (1982) reported the identification of a series of C_{19-30} tricyclic terpanes present in bitumen from the Athabasca oil sands with a cheilanthane 'core', with the branched alkyl side chain substituted at position 14 (Figure S4; F). Based on the molecular ions, in the mass spectra of the hydrocarbons tt-I to –VI, the series included two C_{20} (tt-I and -II), two C_{21} (-III and -IV), one C_{23} (-V) and one C_{24} (-VI) compound (Figure S4). The structures were proposed to possess cheilanthane 'cores', methyl- substituted in the 13-position with a branched alkyl chain substituted in the 14-position. However, the exact position of the alkyl chain, or carboxylated side chain in the original acids, is a subject of debate (Cyr and Strausz, 1983).

3.1.4. Tetracyclic and Pentacyclic Acids

The presence of tricyclic diamondoid acids in OSPW was proposed to be, at least partly, due to the biotransformation of the corresponding hydrocarbons (Rowland et al., 2011b). Pentacyclic diamondoid hydrocarbons, such as diamantane and alkyl diamantanes have also

been reported in various crude oils and were reported to be much more resistant to biodegradation compared to the adamantane homologues (Wang et al., 2006).

However, some pentacyclic diamondoid acids have also been detected in OSPW (Rowland et al., 2011d). The occurrence of these acids was suggested to occur from the biodegradation of the corresponding alkyl diamantanes and that their presence was evidence for biodegradation (Rowland et al., 2011d). Following the successful conversion and identification of the alkyl adamantanes herein (and thus alkyl adamantane acids), the hydrocarbons of both reduced samples #1 (reduced esters of NA) and #2 (reduced free acid NA) were examined for isomers of penta- and tetracyclic 'diamondoids'.

Reference spectra for diamantane hydrocarbons, whilst fewer than those of the adamantanes, are as distinctive, due the stability of the cage-like core, (e.g. displaying base peak ions due to the loss of an alkyl radical and intense molecular ions for isomers with the alkyl substituent on a secondary carbon position). Comparison of the reference mass spectra of 1-, 3- and 4-methyldiamantanes and their GC elution order (Wingert, 1992; Wang et al., 2013), allowed the identification of all three isomers in sample #2 and of 3-methyldiamantane in sample #1 (Figure S5; B).

Extracted ion monitoring of the key fragment ions revealed a series of isomers assigned as alkyl diamantanes (diA-I to diA-XXV) ranging from C_{15-18} based on the observed molecular ions (Figure S5). Interpretation of the mass spectra of peaks diA-I to -XXV (examples are shown in Figure S5; B-H), resulted in the assignment of isomers of ethyl- (D), dimethyl- (E), ethylmethyl- (F and G) and dimethylethyl- (H) diamantanes. These results provided strong supporting evidence for the array of highly substituted alkyl diamantane acids previously speculated to be present in OSPW (Rowland et al., 2011d).

Tetracyclic acids were also tentatively assigned previously based on mass spectral interpretation of the spectra of the methyl esters (Rowland et al., 2011d). The tetracyclic acids were proposed to possess ring-opened diamantane structures (Figure S6; A). However, comparison of the retention positions and mass spectra of synthetic reference acids with those of NA within OSPW, did not confirm their identification (Rowland et al., 2011a).

Series of peaks were observed in the hydrocarbon products obtained from both samples #1 and #2 herein, with spectra with molecular and base peak ions consistent with C_{14-18} tetracyclic hydrocarbons, and some additional C_{13} isomers in the reduced sample #2 (Figures S7 and S8). The mass spectra were very similar to those of alkyl adamantanes and alkyl diamantanes, dominated by a base peak ion presumably corresponding to the loss of the largest alkyl substituent and suggesting a highly stable core (Figures S7 and S8). These hydrocarbons were presumed to originate from tetracyclic acids, such as those previously tentatively assigned (Rowland et al., 2011d). Subsequent examination of data for the original acid methyl esters indeed showed isomers across the same carbon number range, with mass spectra similar to those previously reported in other OSPW samples (Figure S9) (Rowland et al., 2011d).

The lowest carbon number isomers (C_{13-14}) displayed base peak ions at *m/z* 161 (Figure S7; B-D). Assuming the compounds possess 'adamantanoid-like' structures, this indicated a C_{12} 'core'; *m/z* 161 (100 %) corresponding with a $C_{12}H_{17}^{+}$ highly stable cation. The only alkyl (tetracyclic) 'adamantanoid' structures with a C_{12} core, previously assigned in crude oils, are alkyl 2,4-cyclopentano-adamantanes (Petrov, 1987). Other stable, non-adamantanoid tetracyclic structures, such as bridged or highly condensed cyclopentyl structures would not allow for isomers with carbon numbers as low as C_{13} and the few reference mass spectra available were significantly different (Vorob'eva et al., 1986).

Analysis of the reduced acid, hydrocarbon products herein therefore provides the first evidence for a firmer assignment of the tetracyclic acids as possessing cyclopentano-adamantane cores e.g. 2,4-cyclopentano-adamantane acids (Figure S6; B and C), at least for some of the higher carbon number homologues. Subsequent re-examination of the unfractionated OSPW NA as their acid methyl esters, did indeed reveal esters with mass spectra consistent with those of the corresponding cyclopentano-adamantane acids (Figure S9).

3.1.5. Monoaromatic Acids

Fraction 5 from the argentation fractionation of sample #1, was selected for conversion to the hydrocarbons since it was rich in aromatic acids, as indicated by infrared spectroscopy (FTIR), strong UV absorption and adsorption to silver ions in argentation chromatography (Jones et al., 2012). The 'aromatic' acids have been shown to have similar acute toxicity to the 'alicyclic' acids but only produce a weak estrogenic effect in zebrafish larvae (Scarlett et al., 2013).

Rarely cited early investigations of petroleum NA, also showed that aromatic acids were present (Knotnerus, 1957), but the use of the term 'naphthenic' to describe the acids appears to have led most recent workers to overlook the relevance of the aromatic species. Even with this realisation (Rowland et al., 2011c; Jones et al., 2012; Bowman et al., 2014), very few aromatic acids have been firmly identified in OSPW to date.

However, using the mass spectral characteristics of aromatic hydrocarbons to aid assignments herein, series of peaks were assigned as monoaromatic species (Figure S10). The base peak ions in the mass spectra of isomer ma-I to -IV (e.g. *m/z* 105, 119, 133) suggested these consisted mainly of di-, tri- and tetramethylalkylbenzenes (McLafferty, 1963). The presence of many fragment ions (e.g. *m/z* 175, 161 and 147) suggested they did not possess straight n-

alkyl chain substituents, since the spectra were dissimilar from those reported (Ji-Zhou et al., 1993). The lack of intense ions at *m/z* 106, 120 and 134 (from γ-hydrogen transfer as opposed to benzylic or β-cleavage) also implied that either, the alkyl chain was not methyl-substituted at the γ-position or, that both ortho- positions on the benzene ring were substituted (Sinninghe Damsté et al., 1988). Some of the mass spectra closely resembled those reported for monoaromatic hydrocarbons identified as alkylbenzenes obtained from artificially matured melanoidins (Larter et al., 1981). This evidence implied that the corresponding alkylbenzene alkanoic acids were OSPW NA.

Since alkylbenzenes are common pollutants and conceivably might also be hydrocarbon contaminants in the OSPW, the corresponding acid methyl esters were searched for in the esterified OSPW NA, based on the expected retention differences observed between the acid methyl esters and the hydrocarbons (Figure S11). The acids (methyl esters) were indeed present. The similarity in mass spectra between the alkylbenzene hydrocarbons and acids (e.g. Figure S12) indicated that the carboxylate moiety was at the end of an alkyl side chain and not directly substituted onto the benzene ring, consistent with the current understanding of bacterial degradation of branched alkyl hydrocarbons (Misiti et al., 2014).

Recently, the methyl esters of C_{11} indane-2-acetic acid and tetralin-2-carboxylic acids were identified, following derivatization, in oil sands composite tailings pore water (Bowman et al., 2014). Such low molecular weight acids were not present in the OSPW aromatic fraction of sample #1 under study herein and hence were not observed in the hydrocarbon reduction products. Nonetheless, series of larger $C_{14\text{-}20}$ branched alkyl indanes and tetralins were tentatively identified (mb-I-VI) by comparison of the spectra in the NIST mass spectral library and with available literature mass spectra (Figure S13) (Booth et al., 2006). These identifications imply that the corresponding indane and tetralin alkanoic acids, not known previously, were present in OSPW.

Numerous tricyclic monoaromatic acids were present in OSPW NA, though the only acid to be assigned to date is dehydroabietic acid (DHAA) (Jones et al., 2012). One compound (tm-I) with a mass spectrum which was similar to that of the corresponding hydrocarbon, dehydroabietane, was identified in the OSPW hydrocarbons obtained from reduction of the 'aromatic' fraction of sample #1 (Figure S14; A and B). However, the spectrum of tm-I was even more similar to that of the C_{19} and isomeric C_{20} hydrocarbons 13,14-dimethyl- and 13-methyl-14-ethylpodocarpa-8,11,13-triene (Figure S14; C and D) (Azevedo et al., 1990). This was supported by a subsequent retro-examination of the aromatic acid methyl esters of this fraction, which failed to reveal the presence of DHAA methyl ester in this OSPW sample (*cf.* unlike a different OSPW sample examined by Jones et al. (2012)).

Identification of the corresponding alkyl substituted podocarpa-8,11,13-triene acid has not been previously reported in OSPW NA. However, Azevedo et al. (1994) reported the presence of a series of $C_{19\text{-}30}$ tricyclic C-ring monoaromatic carboxylic acids possessing the same structure, with the alkanoate chain in the 14-position in Tasmanian tasmanite (marine type shale).

3.1.6. Diaromatic Sulphur-Containing Acids

Fraction 7 from the fractionation of sample #1, was selected because it is a sulphur-rich, aromatic fraction of OSPW NA for conversion to hydrocarbons (West et al., 2014). The latter authors West et al. (2014) tentatively assigned five $C_{16\text{-}18}$ diaromatic sulphur-containing acids as possessing methyl substituted dibenzothiophene or dimethylnaphthothiophenes (or dimethyldibenzothiopyrans) with methyl branched propanoate acid side chains. Despite comparison of the GC×GC retention times and mass spectra with those of reference dibenzothiophene acid methyl esters and rigorous mass spectral interpretation, none were firmly identified in that study.

Examination of the 'aromatic/sulphur'-containing fraction of acid methyl esters analysed herein by GC×GC-MS indeed revealed the five $C_{16\text{-}18}$ isomers reported by West et al. (2014). Following reduction, the corresponding sulphur-containing hydrocarbons were detected, possessing mass spectra similar to those of the original acid methyl esters (e.g. dominated by base peak ions at *m/z* 197, 211 and 225), corresponding to the loss of $\cdot C_3H_7$ (Figure 3 and S15). Application of a CLIC expression (Figure 3; B) enabled clear mass spectra to be obtained for interpretation and comparison with reference spectra and compounds. The only structure possible for the alkanoate side chain of these isomers, consistent with the loss of M-87 and the absence of a radical cation produced from a McLafferty rearrangement in the mass spectra of the methyl esters (West et al., 2014), along with the consistent loss of $\cdot C_3H_7$ by benzylic cleavage in the mass spectra of the hydrocarbons, was that of a dibenzothiophene (or naphthothiophene) containing an isobutyl side chain. The retention position and mass spectrum of synthesised 4-isobutyldibenzothiophene matched that of the dbt-I isomer, confirming its identification (Figure 3; C and D), and by inference, the identification of dibenzothiophene-4-isobutanoic acid in OSPW NA.

West et al. (2014) also reported the presence of sulphur-containing acids with 8 and 9 double bond equivalents (DBE), eluting slightly earlier than those compounds possessing 10 DBE now identified as dibenzothiophene acids. Examination of the F7 hydrocarbon product herein, revealed a series of isomers with spectra displaying molecular ions consistent with diaromatic sulphur-containing hydrocarbons with 8 DBE (e.g. *m/z* 214, 228, 242 and 256), eluting slightly earlier than the 10 DBE dibenzothiophenes (e.g. isomer nat-1 in Figure 3; A).

The mass spectra of these isomers (nat-1 to -V) were dominated by intense base peak ions at *m/z* 171 and 185, with very few fragment ions observed (Figure S16). This indicated that the compounds were highly condensed, with the base peak ion most likely formed via benzylic cleavage of an alkyl substituent. Comparison of the mass spectrum of the isomer nat-I, with

reference spectra in the NIST MS Library, showed that the spectrum was similar to that of 2-methyl-2H-naphtho[1,8-bc]thiophene (Figure S16; A and B). The NIST mass spectrum showed a loss of M-15, attributed to fragmentation of the methyl substituent, via benzylic cleavage, as proposed previously for the fragmentation of alkyl 2H-naphtho[1,8-bc]thiophenes (Figure S16; A) (Porter, 1985).

The mass spectrum of nat-I displayed a similar fragmentation pattern and ions to that of the NIST reference spectrum. However, with the base peak ion of nat-I corresponding to the loss of M-43 instead, this was attributed to the loss of a propyl group $\cdot C_3H_7$ (Figure S16; B). The mass spectra of naphtho[1,8-bc]thiophene acids would be expected to display similar mass spectral fragmentation patterns (due to the stability of the condensed structure) with additional radical cations due to McLafferty rearrangement of the alkanoate side chain, involving the hydrogen on the carbon adjacent to the sulphur atom. Subsequent re-examination of the OSPW F7 NA methyl esters indeed revealed a series of peaks with mass spectra displaying fragmentation patterns similar to those of the nat-I to -V hydrocarbons, with additional even mass ions, corresponding to M-74, M-88 and M-102 ions (Figure S17), attributed to neutral losses from hydrogen rearrangement on the alkanoate side chain. These observations, coupled with the spectral interpretation; similarity with the reference spectrum (Figure S16; A and B); retention position relative to the dibenzothiophenes and precedent for the presence of sulphur-containing acids with 9 DBE confirmed by GC×GC with sulphur chemiluminescence detection and high resolution mass spectrometry (West et al., 2014), led to the tentative assignment of naphtho[1,8-bc]thiophene acids in OSPW NA herein.

Sulphur-containing hydrocarbons possess the same nominal mass as some aromatic hydrocarbons and could be misassigned when analysed by unit resolution mass spectrometric techniques (Hegazi and Andersson, 2007). However, interpretation and comparison of the

475 mass spectra of such hydrocarbons, clearly ruled them out as possibilities for those discussed
476 above.

477 4. Conclusions

478 The identifications presented herein of individual hydrocarbons and thus acids in OSPW,
479 included alicyclic, aromatic and sulphur-containing NA. The assignments were consistent
480 with, but expanded upon, previous evidence of such acids in other matrices, identified as the
481 esters (Rowland et al., 2011b; Rowland et al., 2011d; West et al., 2014; Wilde et al., 2015)
482 and were supported by the identification of multiple isomers of each acid type. The acids
483 identified can perhaps be used to better inform environmental monitoring programs as well as
484 for geochemical and toxicity studies. The same approach may have wide applicability to
485 other polluted waters and functionalised organic compounds in sediments and petroleum.

486 Supplementary Material

487 Mass spectra of all identified hydrocarbons and a summary of all identifications made are
488 provided in the Supplementary Material (Table S4 and Figures S1-S17).

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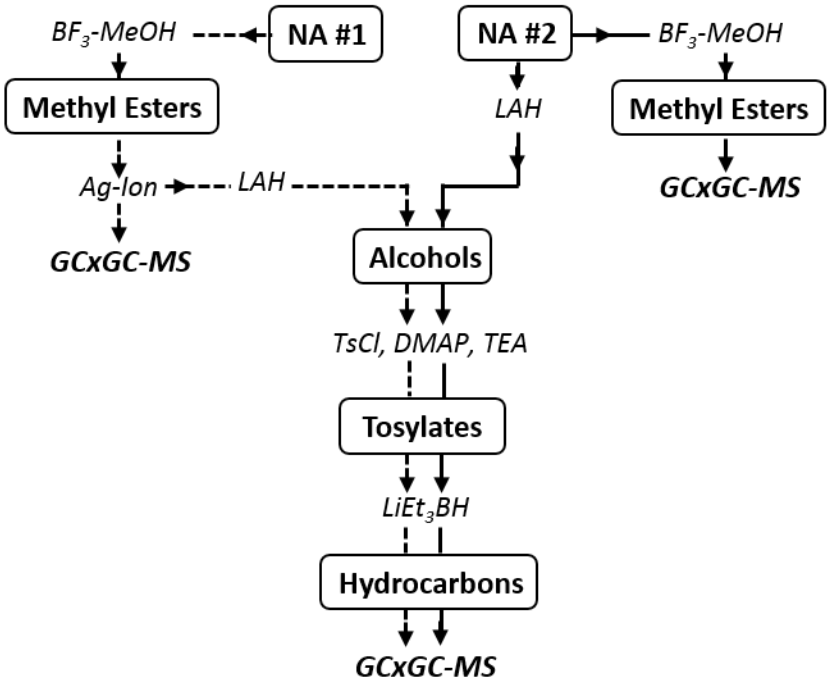
Scheme 1: Route of conversion of the OSPW NA samples #1 and #2, to the corresponding hydrocarbons.



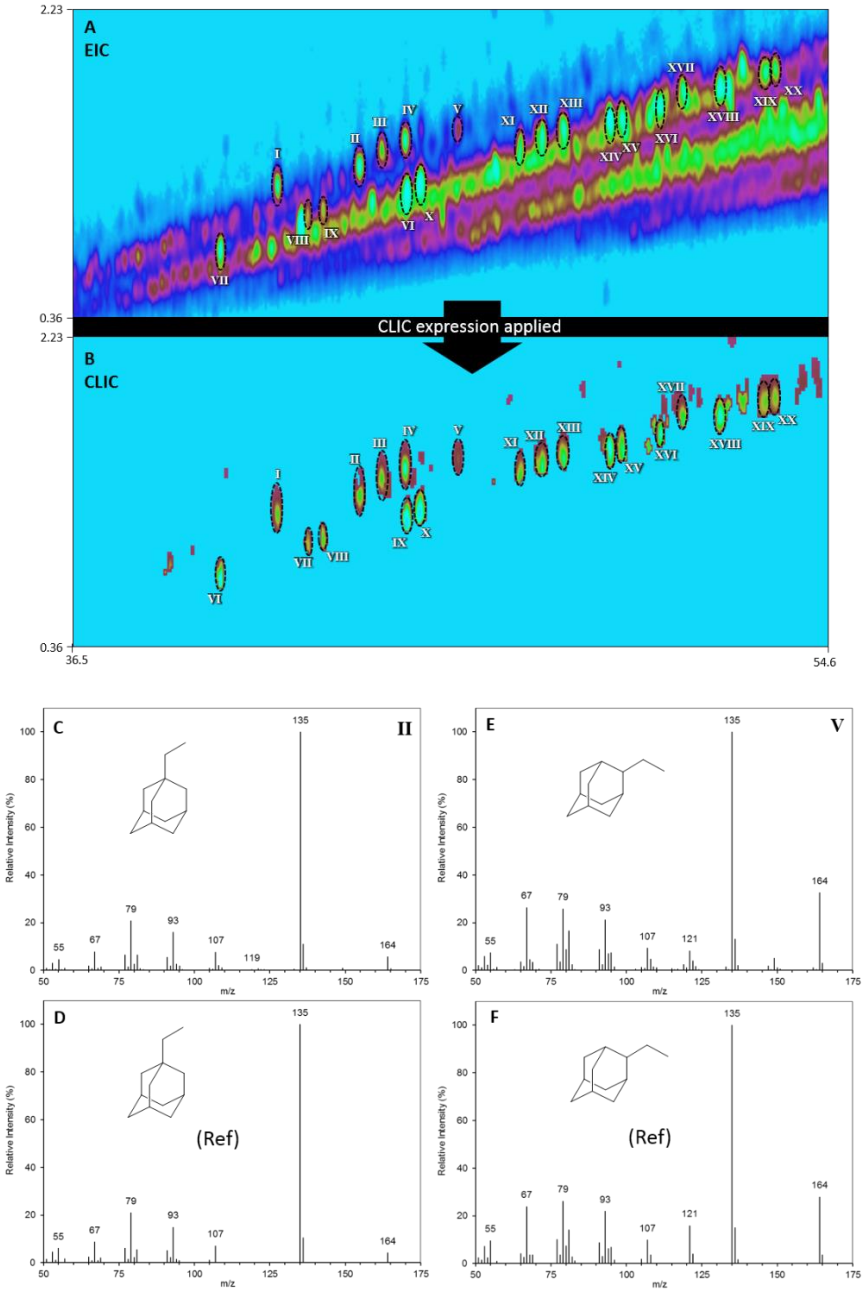


Figure 1: Comparison of (A) an EIC (*m/z* 135, 149, 163 and 178) and (B) a simplified chromatogram after a CLIC expression was applied of the reduced unfractionated OSPW sample (#2), clearly showing the presence of three dimethyl- (Ad-I, -III and -IV), (C-F) two ethyl- (-II and -V), eight trimethyl- (-VI-VIII, -X-XIII and -XVII) and seven ethylmethyl-adamantane isomers (-IX, -XIV-XVI and -XVIII-XX), based on comparison with reference mass spectra and known elution order (Polyakova et al., 1973; Wingert, 1992; Wang et al., 2013).

Please note: This is an accepted, pre-publication, manuscript. Readers are advised to consult the final, published version: doi: 10.1016/j.orggeochem.2017.09.004

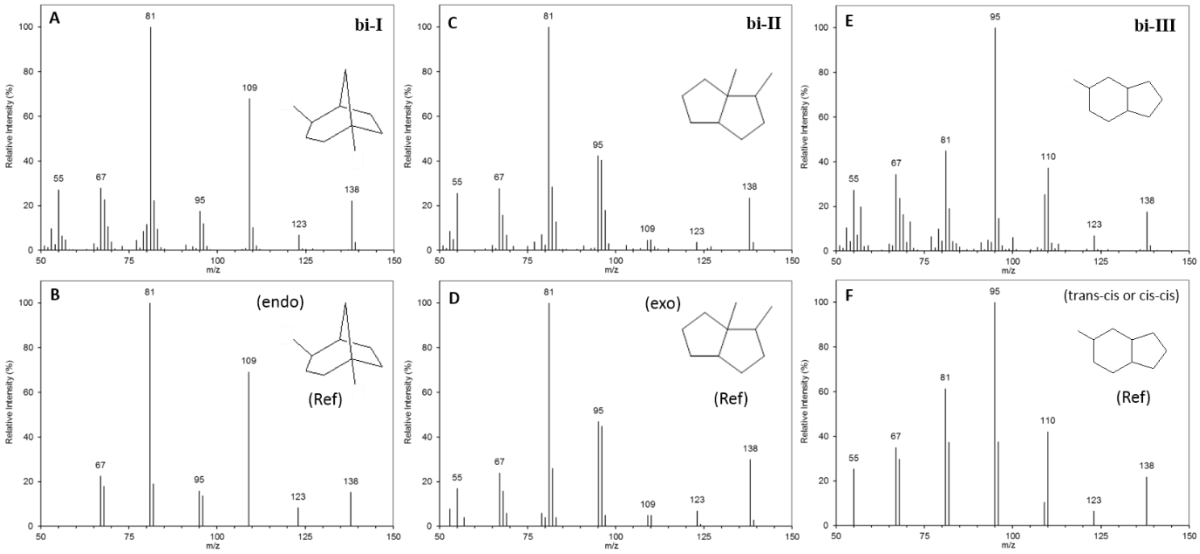


Figure 2: Identification of bridged and fused bicyclanes by mass spectral comparison with reference spectra and elution order (Denisov et al., 1977a; Denisov et al., 1977b; Denisov et al., 1977c).

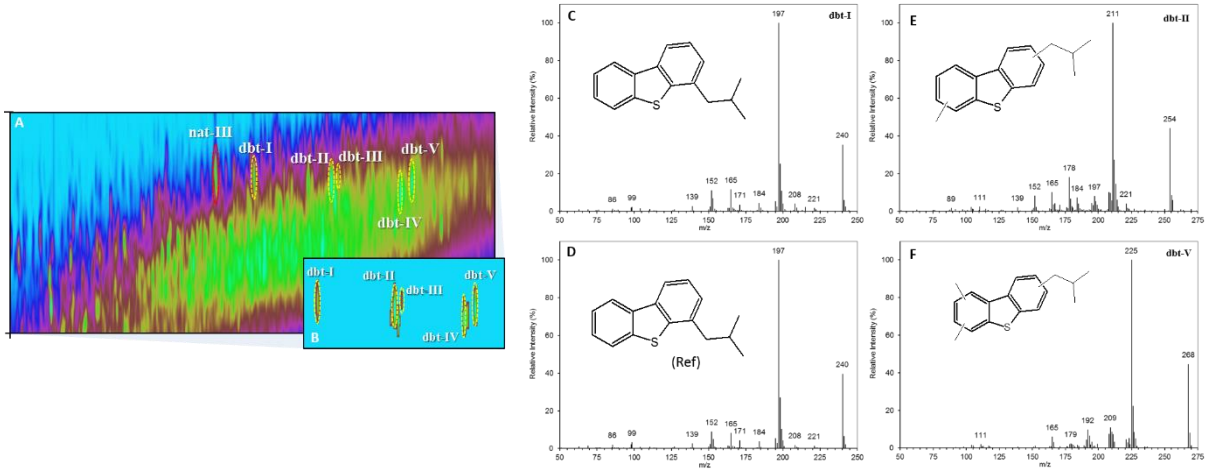


Figure 3: (A-B) TIC and CLIC EIC of reduced 'aromatic/sulphur' fraction (sample #1), showing isomers dbt-I to -V assigned as the reduced hydrocarbons of the five methyl esters reported by West et al. (2014). (C) Identification of dbt-I after comparison with (D) synthesised 4-isobutyldibenzothiophene and (E-F) assignment of dbt-II and –V as methyl- and dimethyl- isobutyldibenothiophenes.

SUPPORTING INFORMATION FOR PUBLICATION

Naphthenic acids in oil sands process waters: Identification by conversion of the acids or esters to hydrocarbons

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SUPPORTING INFORMATION EXERPIMENTAL DETAILS

4.1. GCxGC-MS

The GC×GC-MS conditions set A involved the primary oven programmed from 30 °C, held for 1 min, then heated to 120 °C at 5 °C/min, to 220 °C at 0.8°C/min, to 280 °C at 5 °C/min and to 320 °C at 10 °C/min and then held for 10 min. The secondary oven was programmed to track the primary oven at 40 °C above. The hot jet was programmed to start 30 °C above the primary oven temperature until 150 °C, it was then ramped to 260 °C at 1.3 °C/min and then to 400 °C at 4 °C/min. The modulation period was set 4 and 6 s. The GC×GC-MS conditions set B involved the primary oven programmed from 40 °C, held for 1.3 min, then heated to 200 °C at 1 °C/min, to 280 °C and 5 °C/min and to 320 °C at 10 °C/min and then held for 5 min. The secondary oven was programmed to track the primary oven at 20 °C above. The hot jet was programmed to start 20 °C above the primary oven and finish 100 °C above the primary oven over the period of the run; programmed from 60 °C, held for 1 min, then heated to 200 °C at 1.1 °C/min, to 280 °C at 3 °C/min and then to 400 °C at 5 °C/min. The modulation period was set at 4 and 6 s.

4.2. Derivatisation and fractionation of sample #1

A concentrated naphthenate solution, extracted from oil sands process-affected water (OSPW), which had undergone a prior clean-up procedure involving weak anion exchange chromatography, was received from Environment Canada, Burlington, CA. The extraction and clean-up procedure was developed and reported by Frank et al. (2006).

The free NA (537 mg), extracted from the concentrated OSPW naphthenate solution as described by Jones et al. (2012), was dissolved 14 % BF3-methanol complex solution (Sigma Aldrich) and heated at 70 °C for 3 hours. The methylated solution was washed with water (Chromasolv® HPLC Grade, Sigma Aldrich) and the methyl esters extracted with hexane (HPLC Grade, Rathburns Chemical Ltd.). The methyl ester solution was dried over anhydrous Na_2SO_4 (≥ 99.0 %, Sigma Aldrich), filtered and evaporated to dryness under N_2 at 40 °C.

The large scale fractionation was performed twice, on two quantities of sample #1. The mass of OSPW NA methyl esters loaded onto the phase in the first fractionation was 296 mg, and 300 mg in the second fractionation. A summary of the fractions for each fractionation is given in Tables S1 and S2.

TABLES

Table S-1: Summary of the fractions collected in the first Ag-Ion fractionation of the OSPW NA methyl esters.

Fraction	Eluent	Mass of eluate / mg
Wash	Hexane	0.4
1	100 % hexane	20.1
2	100 % hexane	76.8
3	100 % hexane	7.4
4	100 % hexane	1.7
5	5 % diethyl ether : 95 % hexane	61.3
6	5 % diethyl ether : 95 % hexane	30.8
7	5 % diethyl ether : 95 % hexane	9.2
8	10 % diethyl ether : 90 % hexane	11.5
9	100 % diethyl ether	29.6
10	100 % methanol	52.1
	Total	300.9
	Total (excluding F10)	248.8

Table S-2: Summary of the fractions collected in the second Ag-Ion fractionation of the OSPW NA methyl esters.

Fraction	Eluent	Mass of eluate / mg
Wash	Hexane	0.3
1	100 % hexane	7.7
2	100 % hexane	96.7
3	100 % hexane	16.7
4	100 % hexane	2.1
5	5 % diethyl ether : 95 % hexane	50.0
6	5 % diethyl ether : 95 % hexane	38.2
7	5 % diethyl ether : 95 % hexane	12.9
8	10 % diethyl ether : 90 % hexane	15.1
9	100 % diethyl ether	36.1
10	100 % methanol	222.9
	Total	498.4
	Total (excluding F10)	275.5

Table S-3: Summary of the masses and yields for the conversion of the Ag-Ion fractions 2, 5 and 7 of the derivatised OSPW NA and of the underivatised, unfractionated OSPW NA samples, to hydrocarbons.

Starting Reactant		Starting mass and yield / mg (approx. percentage yield / %)					
		$LiAlH_4$ Reduction		Tosylation		Super-Hydride® Reduction	
Derivatised (# sample number)	Fraction	NA methyl esters / mg	Alcohols / mg	Alcohols / mg	Tosylates / mg	Tosylates / mg	Hydrocarbons / mg
Yes (#1)	2	10†	9.8 (104 %‡)	9.8	13.3 (82 %‡)	12.2	20.7*
Yes (#1)	5	25†	23.0 (97 %‡)	23.0	27.9 (77 %‡)	27.9	17.2*
Yes (#1)	7	10†	9.1 (96 %‡)	9.1	10.5 (74 %‡)	10.5	6.7*
No (#2)	Whole	30†	25.0 (88 %‡)	25.0	32.3 (78 %‡)	32.0	-

† approximate masses, as methyl esters

‡ approximate yield based on average molecular weight of acid methyl esters as 250 g/mol in F2, 286 g/mol in F5, 294 g/mol in F7 and 246 g/mol in the whole underivatised OSPW NA

* hydrocarbon product concentrated by N_2 blowdown resulting in evaporative losses; THF and boroxin residue present

- Kuderna-Danish apparatus used for concentration to reduce volatile compound losses, so no weight was recorded

Table S-4: Summary of naphthenic acids within OSPW identified as hydrocarbons after reduction of the esters and analysis by GCxGC-MS

Assigned label	Type	Hydrogen Deficiency of hydrocarbon or NA (z)	Name of hydrocarbon equivalent	Identification
Ad-I	Tricyclic (diamondoid)	-6	1,2-dimethyladamantane	Mass spectral interpretation, Golovkina et al. (1984); mass spectral comparison, Polyakova et al. (1973); GC elution order, Wingert (1992), Wei et al. (2006), Wang et al. (2013); previous assignment of NA in OSPW and comparison of retention position and mass spectra with reference NA, Rowland et al. (2011a), Bowman et al. (2014), Lengger et al. (2013)
Ad-II	Tricyclic (diamondoid)	-6	1-ethyladamantane	
Ad-III	Tricyclic (diamondoid)	-6	cis-2,4-dimethyladamantane	
Ad-IV	Tricyclic (diamondoid)	-6	trans-2,4-dimethyladamantane	
Ad-V	Tricyclic (diamondoid)	-6	2-ethyladamantane	
Ad-VI-VIII, X-XIII, XVII	Tricyclic (diamondoid)	-6	trimethyladamantane isomers	
Ad-IX, XIV-XVI, XVIII-XX	Tricyclic (diamondoid)	-6	ethylmethyladamantane isomers	
bi-I	Bicyclic	-4	1,4-dimethylbicyclo[3.2.1]octane	Mass spectral comparison, Denisov et al. (1977a); GC elution order Piccolo et al. (2010); previous assignment in petroleum NA and comparison of retention position and mass spectra with reference NA, Wilde and Rowland (2015), Wilde et al. (2015)
bi-II	Bicyclic	-4	1,2-dimethylbicyclo[3.3.0]octane	Mass spectral comparison, Denisov et al. (1977c); GC elution order Piccolo et al. (2010); previous assignment of isomers in petroleum NA and comparison of retention position and mass spectra with reference NA, Wilde and Rowland (2015), Wilde et al. (2015)
bi-III	Bicyclic	-4	3-methylbicyclo[4.3.0]nonane	Mass spectral comparison, Denisov et al. (1977b); GC elution order Piccolo et al. (2010); previous assignment of isomers in petroleum NA and comparison of retention position and mass spectra with reference NA, Wilde and Rowland (2015), Wilde et al. (2015)
bi-IV	Bicyclic	-4	2,6-dimethylbicyclo[3.2.1]octane	Mass spectral comparison, Denisov et al. (1977a); GC elution order Piccolo et al. (2010); previous assignment of isomers in petroleum NA, Wilde and

				Rowland (2015) (tentative)
bi-V and VI	Bicyclic	-4	C_{10} dimethylbicyclooctane isomers	Mass spectral comparison, Denisov et al. (1977a) (tentative)
tt-I and II	Tricyclic	-6	C_{20} tricylic terpane; cheilanthane isomers	Mass spectral interpretation and mass spectral comparison, Hall and Douglas (1981), Philp (1985), Chicarelli et al. (1988); previous evidence of terpenoid NA in oil sands, Cyr and Strausz (1983)
tt-III and IV	Tricyclic	-6	C_{21} tricylic terpane; cheilanthane isomers	
tt-V	Tricyclic	-6	C_{23} tricylic terpane; cheilanthane isomer	
tt-VI	Tricyclic	-6	C_{24} tricylic terpane; cheilanthane isomer	
diA-I	Pentacyclic	-10	3-methyldiamantane	Previous assignment of NA isomers in OSPW, Rowland et al. (2011b); mass spectral interpretation, Golovkina et al. (1984) and mass spectral comparison, Kuraš and Hála (1970), Musayev et al. (1983); GC elution order, Wingert (1992), Wang et al. (2013) (higher homologues are tentative)
diA-II	Pentacyclic	-10	ethyldiamantane	
diA-III-IX	Pentacyclic	-10	dimethyl- and ethyldiamantane isomers	
diA-X-XVI	Pentacyclic	-10	ethylmethyldiamantane isomers	
diA-XVII-XXV	Pentacyclic	-10	dimethylethyldiamantane isomers	
tAd-I	Tetracyclic	-8	methyl-2,4-cyclopentano-adamantane	Mass spectral interpretation and evidence of corresponding NA in OSPW, Petrov (1987), Vorob'eva et al. (1986) (higher homologues are tentative)
tAd-II and III	Tetracyclic	-8	ethyl-2,4-cyclopentano-adamantane isomers	
tAd-IV	Tetracyclic	-8	dimethyl-cyclopentano-adamantane	
tAd-V	Tetracyclic	-8	ethylmethyl-cyclopentano-adamantane	
tAd-VI	Tetracyclic	-8	trimethyl-cyclopentano-adamantane	
tAd-VII-IX	Tetracyclic	-8	$C_{16\text{-}18}$ alkylcyclopentano-adamantane isomers	
ma-I-IV	Monocylic monoaromatic	-8	$C_{16\text{-}18}$ alkylbenzene isomers	Mass spectral interpretation and comparison, Larter et al. (1981), Sinninghe Damsté et al. (1988), Ji-Zhou et al. (1993); evidence of corresponding NA in OSPW
mb-I-VI	Bicyclic monoaromatic	-10	$C_{14\text{-}19}$ alkylindane and tetralin isomers	
tm-I	Tricyclic monoaromatic	-12	13-methyl-14-ethylpodocarpa-8,11,13-triene	Mass spectral comparison, Azevedo et al. (1990), Azevedo et al. (1992) and NIST MS library; previous assignment of monoaromatic terpenoid NA in tasmanite, Azevedo et al. (1994)
dbt-I	Sulphur-containing tricyclic diaromatic	-18	4-isobutyldibenzothiophene	Comparison of retention position with synthetic reference hydrocarbon, Wilde (2015); mass spectral interpretation and mass spectral comparison, Andersson et al. (2001), Bobinger et al. (1999), (Andersson and Schade, 2004), Li et al. (2014); previous assignment of NA

				isomers in OSPW, West et al. (2014)
dbt-II and III	Sulphur-containing tricyclic diaromatic	-18	methyl-isobutyldibenzothiophene isomers	Mass spectral interpretation and mass spectral comparison, Andersson et al. (2001), Bobinger et al. (1999), Andersson and Schade (2004), Li et al. (2014); previous assignment of NA isomers in OSPW, West et al. (2014)
dbt-IV and V	Sulphur-containing tricyclic diaromatic	-18	dimethyl-isobutyldibenzothiophene	
nat-I-V	Sulphur-containing tricyclic diaromatic	-16	alkyl 2H-naphtho[1,8-bc]thiophenes isomers	Mass spectral interpretation and comparison, (Porter, 1985), (Hawthone and Porter, 1968) and NIST MS library

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FIGURES

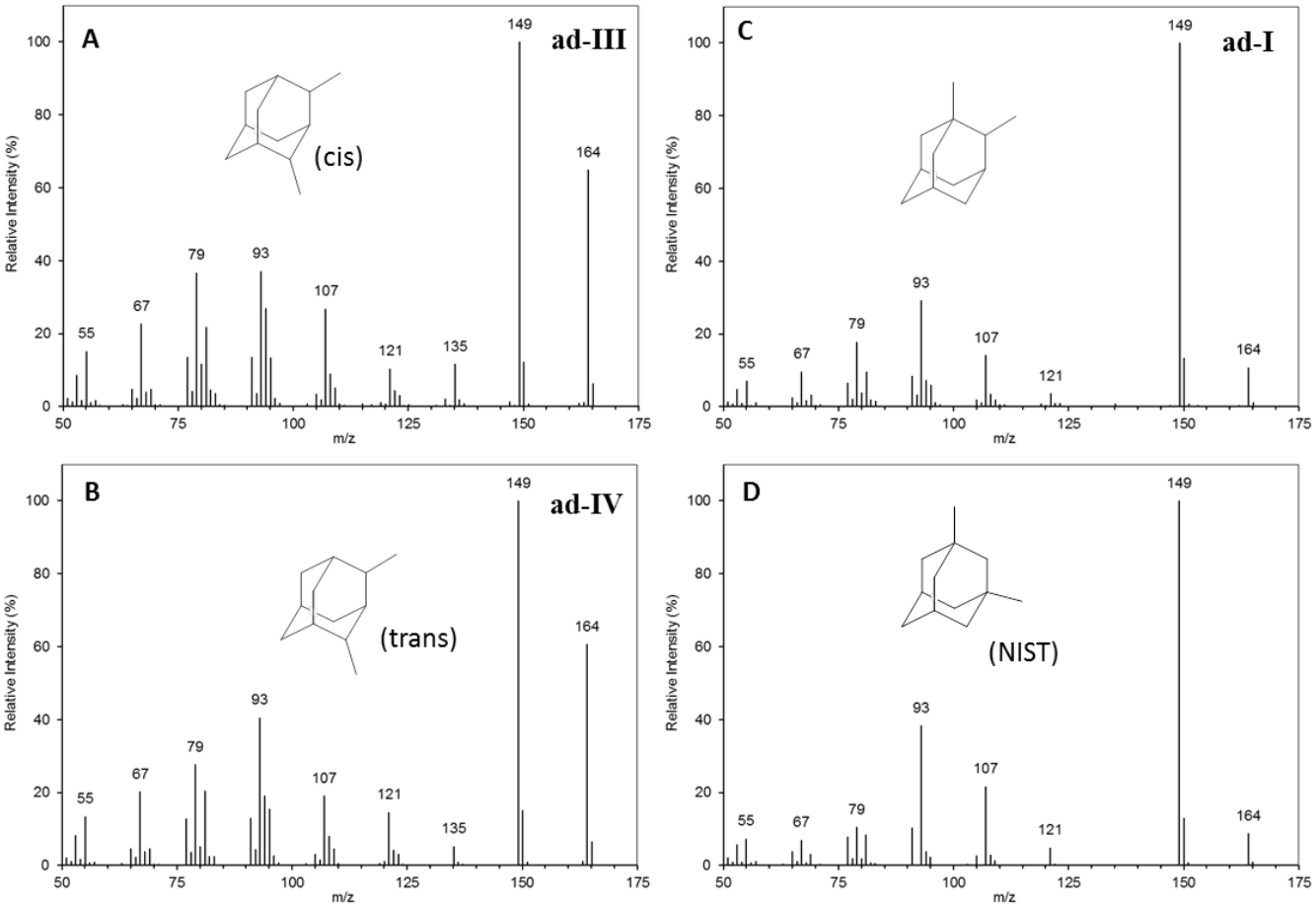

Figure S-1. Mass spectra of isomers ad-I, III and IV (labels refer to components in EIC in Figure 1 in main text) assigned as dimethyladamantanes including (A and B) two isomers of 2,4-dimethyladamantane (III and IV) and (C) 1,2-dimethyladamantane (I) by comparison of the known GC elution order of alkyl adamantanes relative to 1- and 2-ethyladamantane (II and V) and by comparison with (D) the reference mass spectrum of 1,3-dimethyladamantane (Polyakova et al., 1973; Wingert, 1992; Wang et al., 2013).

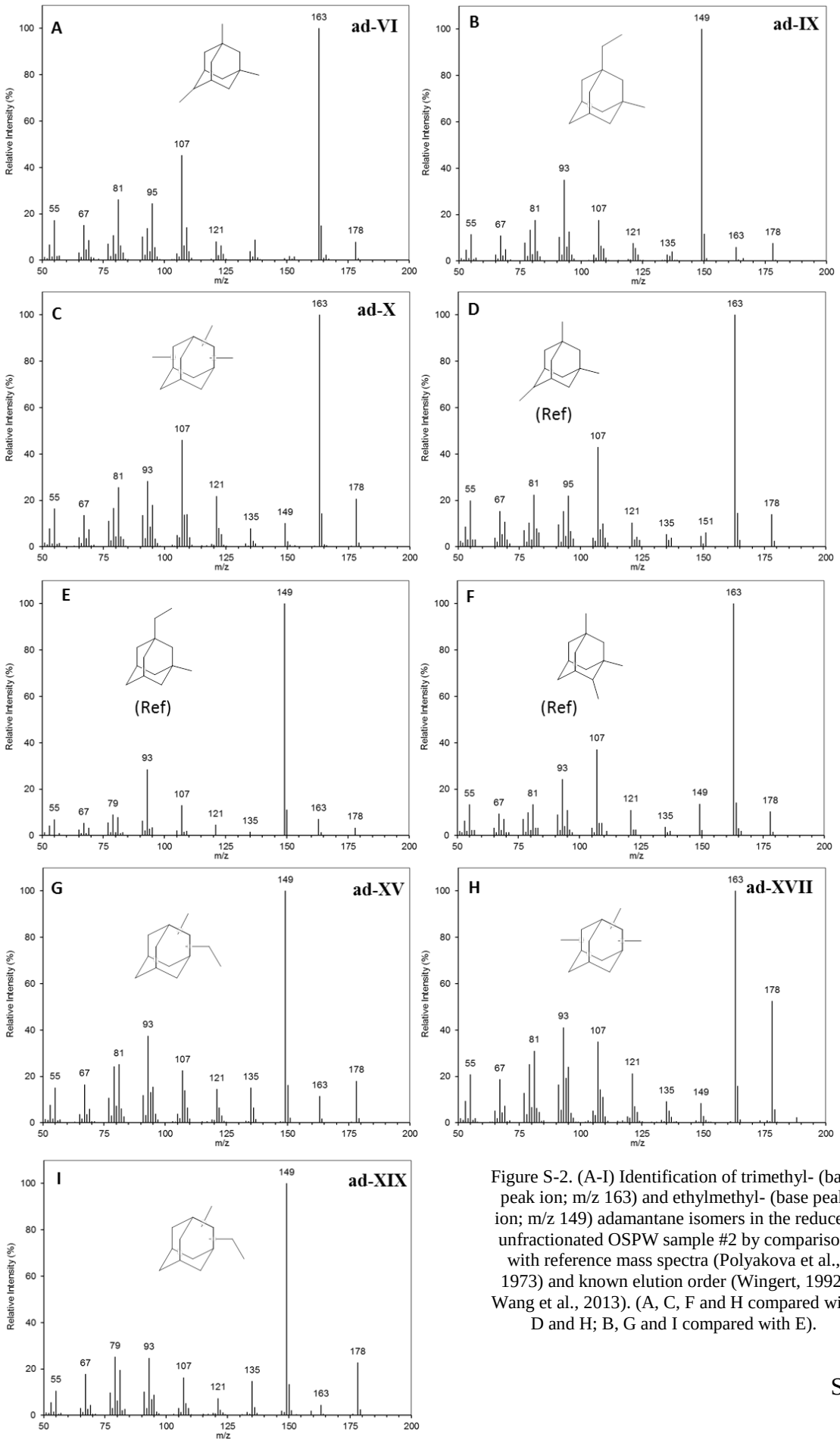

Figure S-2. (A-I) Identification of trimethyl- (base peak ion; m/z 163) and ethylmethyl- (base peak ion; m/z 149) adamantane isomers in the reduced unfractionated OSPW sample #2 by comparison with reference mass spectra (Polyakova et al., 1973) and known elution order (Wingert, 1992; Wang et al., 2013). (A, C, F and H compared with D and H; B, G and I compared with E).

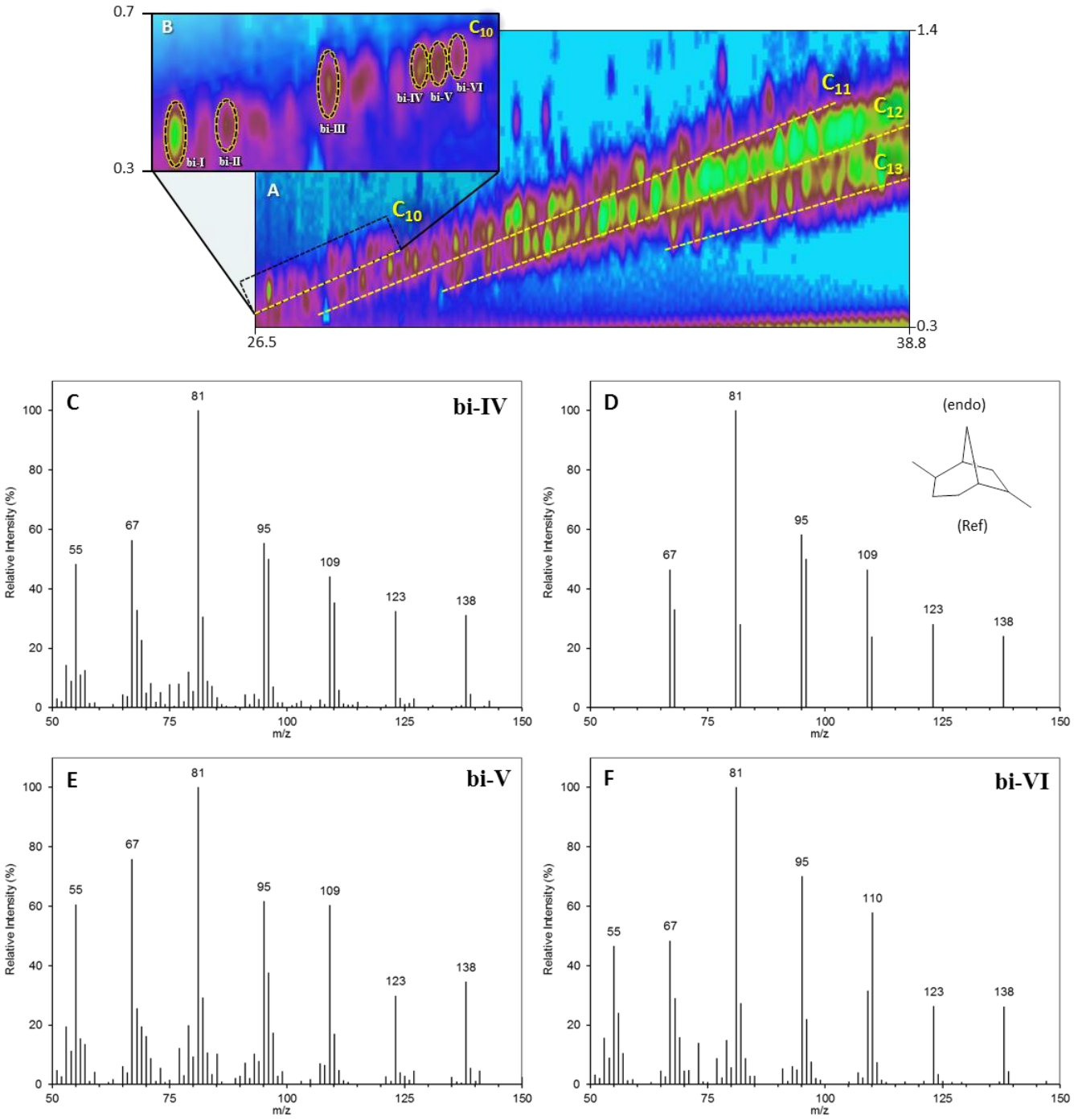

Figure S-3. Mass spectra of C_{10} bicyclanes (bi-IV to VI) in the reduced unfractionated NA (sample #2). (A) Isomer bi-IV assigned after comparison with (B) the reference mass spectrum of endo-endo-2,6-dimethylbicyclo[3.2.1]octane. (C and D) Mass spectra of isomers bi-V and VI which did not match any reference spectra and were significantly different to those within reduced petroleum-derived acids previously reported (Wilde and Rowland, 2015).

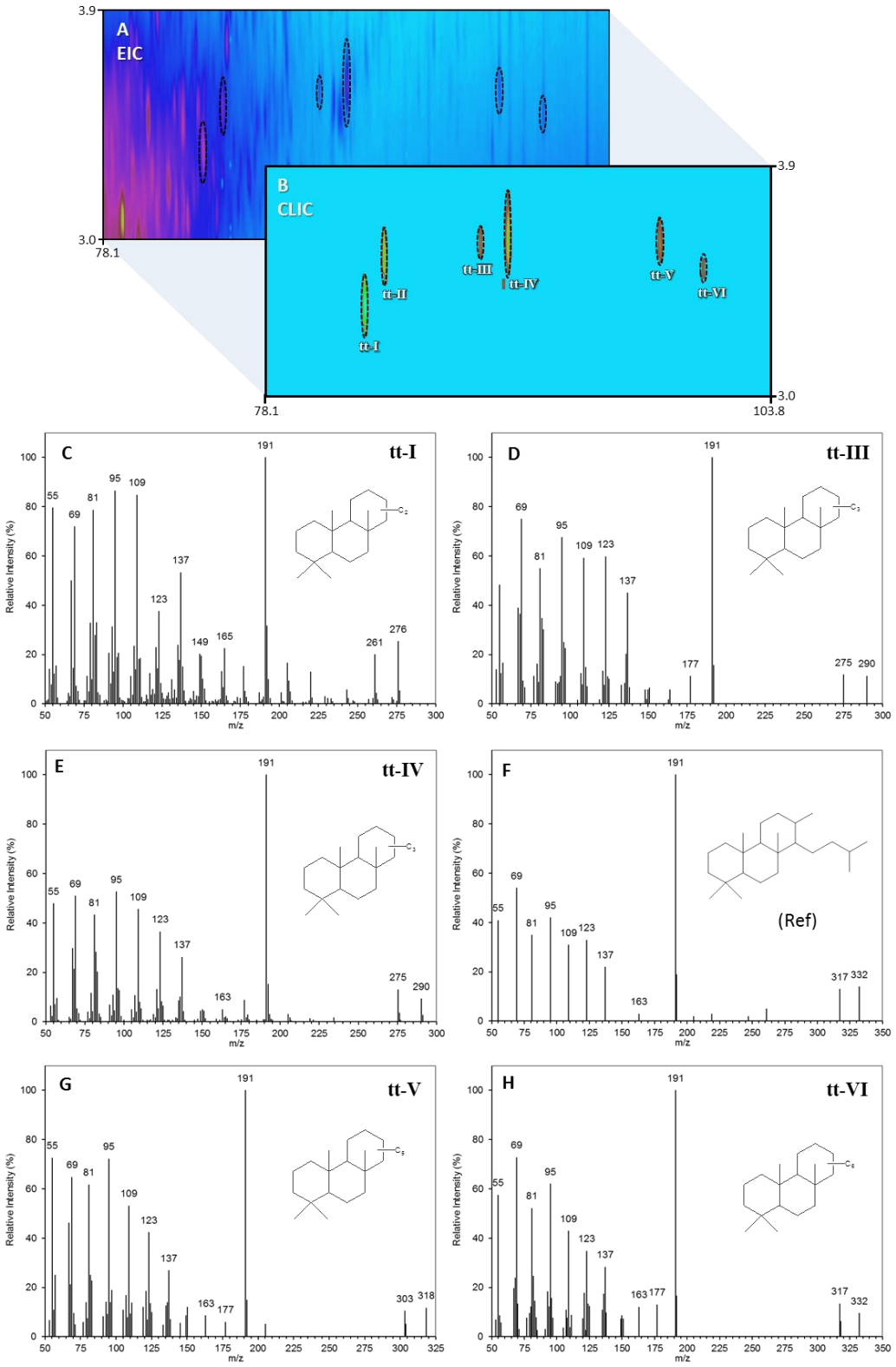

Figure S-4. (A) EIC (m/z 191) and (B) CLIC expression chromatogram revealing series of isomers assigned as (C-H) C_{20} (tt-I and II), C_{21} (tt-III and -IV), C_{23} (tt-V) and C_{24} (tt-VI) tricyclic terpanes by comparison with reference mass spectra (Hall and Douglas, 1981; Cyr and Strausz, 1983; Philp, 1985; Chicarelli et al., 1988). (All mass spectra share similar fragmentation pattern to reference mass spectrum F, particularly H and F).

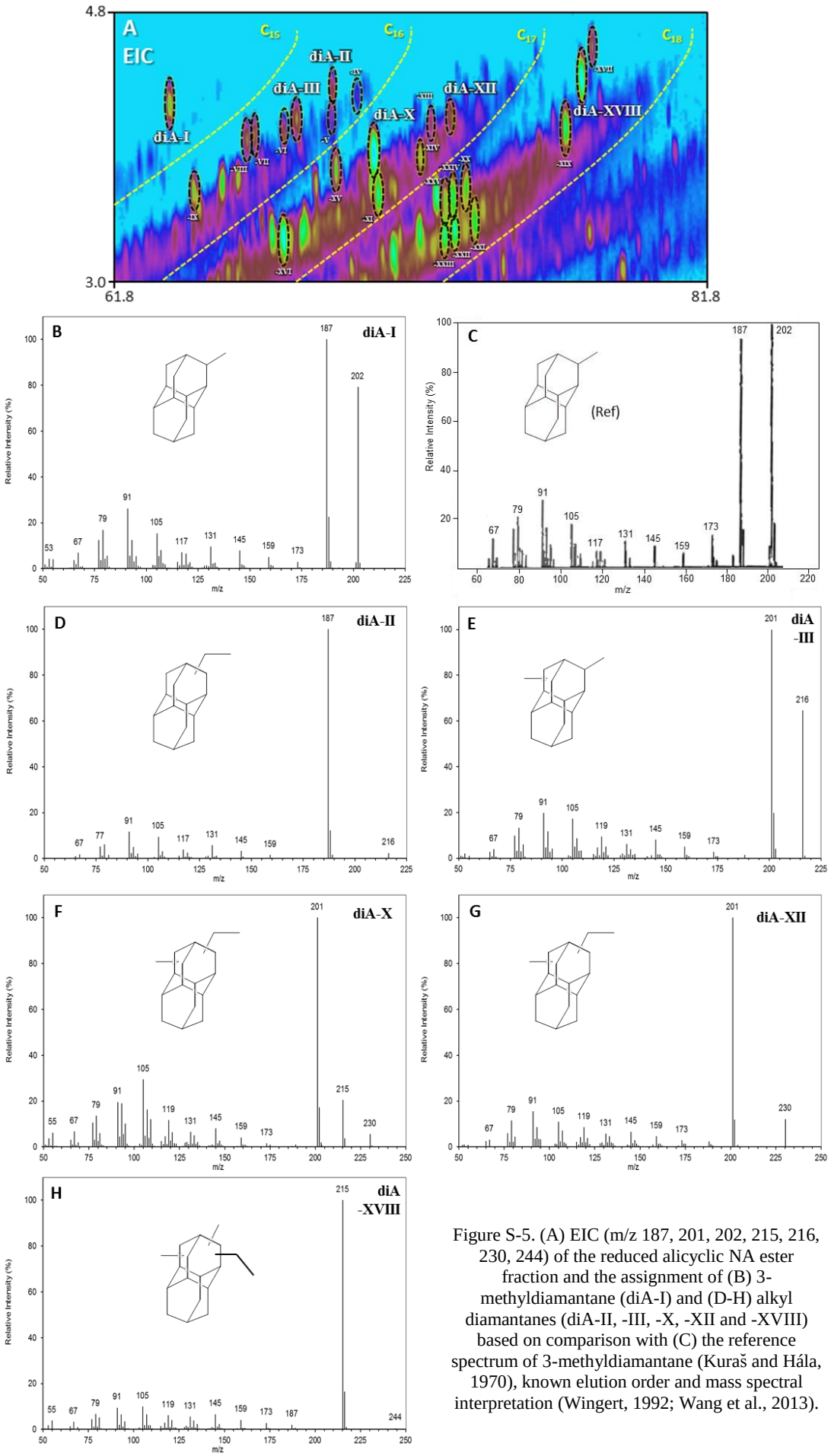

Figure S-5. (A) EIC (m/z 187, 201, 202, 215, 216, 230, 244) of the reduced alicyclic NA ester fraction and the assignment of (B) 3-methyldiamantane (diA-I) and (D-H) alkyl diamantanes (diA-II, -III, -X, -XII and -XVIII) based on comparison with (C) the reference spectrum of 3-methyldiamantane (Kuraš and Hála, 1970), known elution order and mass spectral interpretation (Wingert, 1992; Wang et al., 2013).

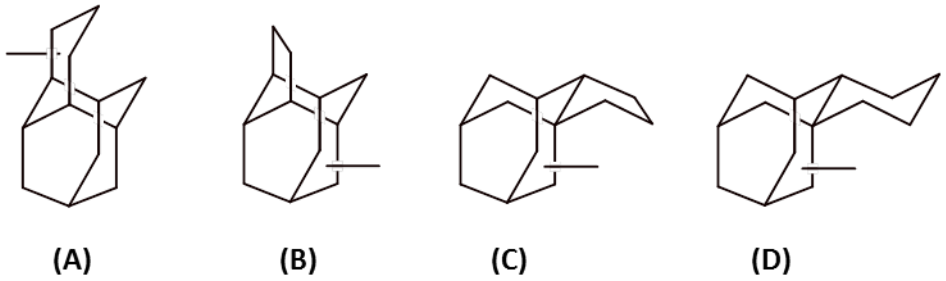

Figure S-6. Examples of substituted tetracyclic diamondoid compounds showing the structure of the skeletal core, including (A) 2,4-cyclohexano-adamantane (tetracyclic ring-opened diamantane), (B) 2,4-cyclopentano-adamantane, (C) 1,2-cyclopentano-adamantane and (D) 1,2-cyclohexano-adamantane.

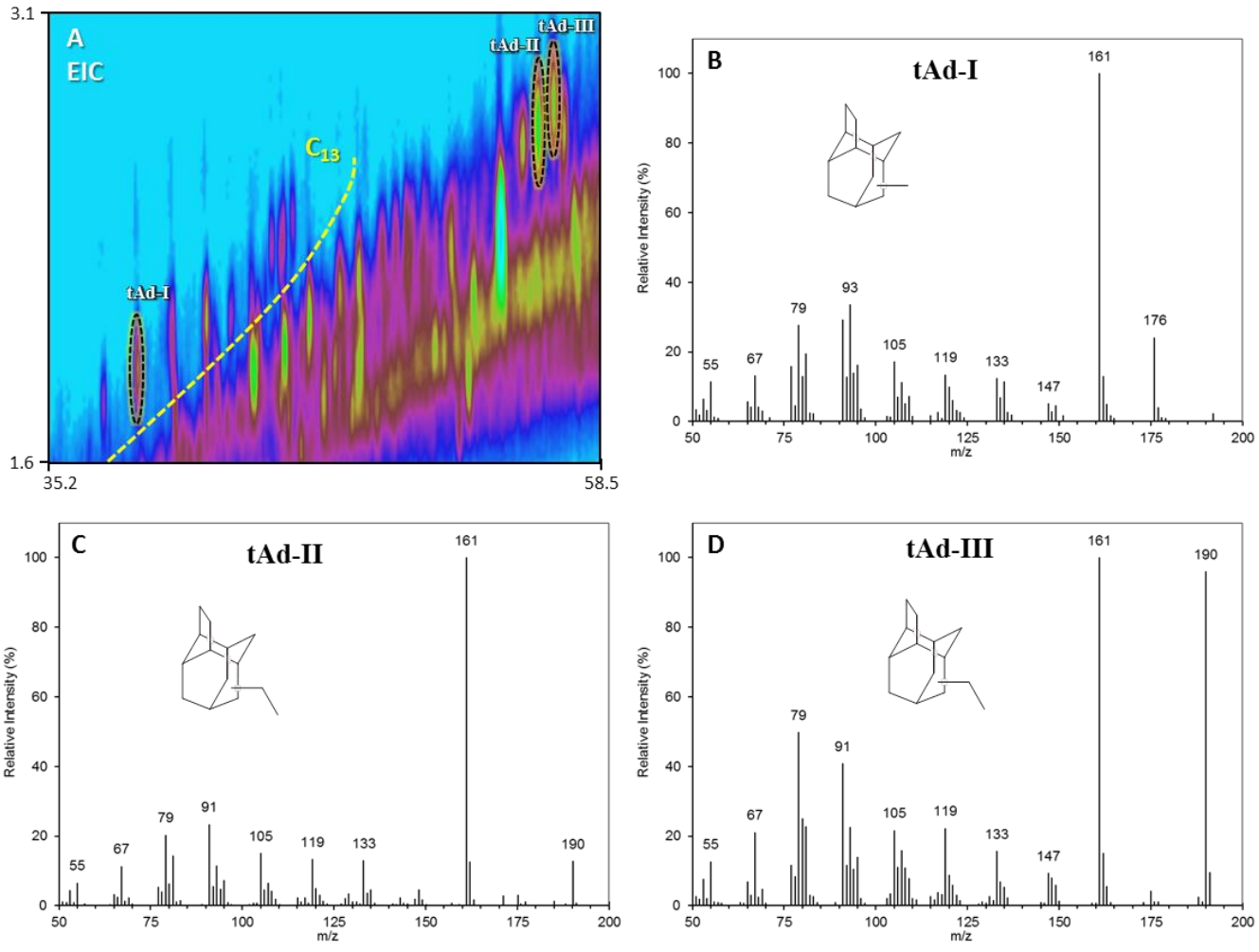

Figure S-7. (A) EIC (m/z 161, 175, 176, 189, 190, 203, 217) of the reduced sample #2, showing components tAd-1 to –III with (B-D) mass spectra containing molecular ions corresponding to C_{13} and C_{14} tetracyclic hydrocarbons, tentatively assigned as alkyl 2,4-cyclopentano-adamantanes.

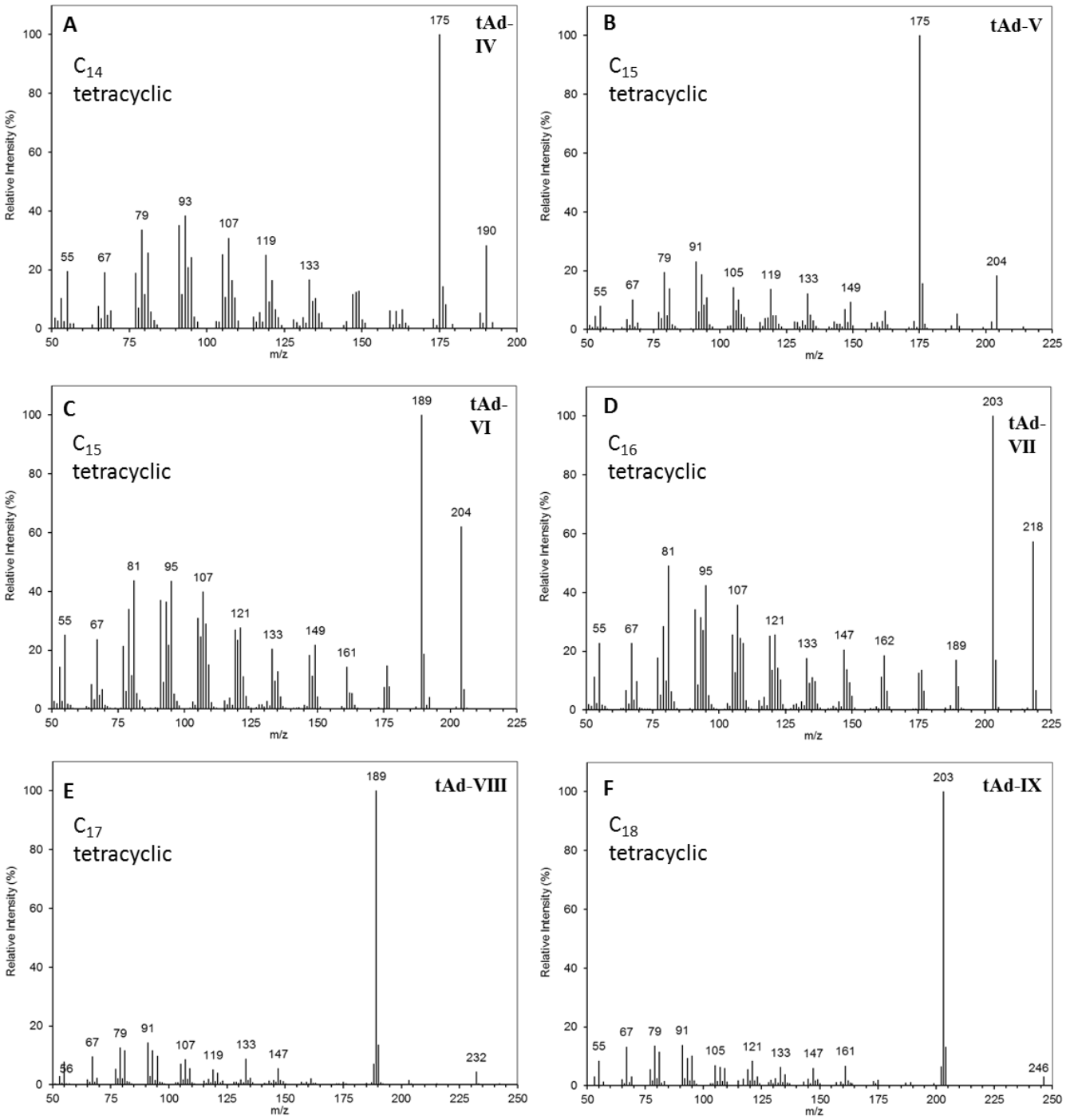

Figure S-8. (A-F) Mass spectra of C_{14-18} alkyl tetracyclic hydrocarbons (isomers tAd-IV to – IX) in the reduced sample #2, postulated to possess adamantanoid structures (e.g. Figure S-6; A-D).

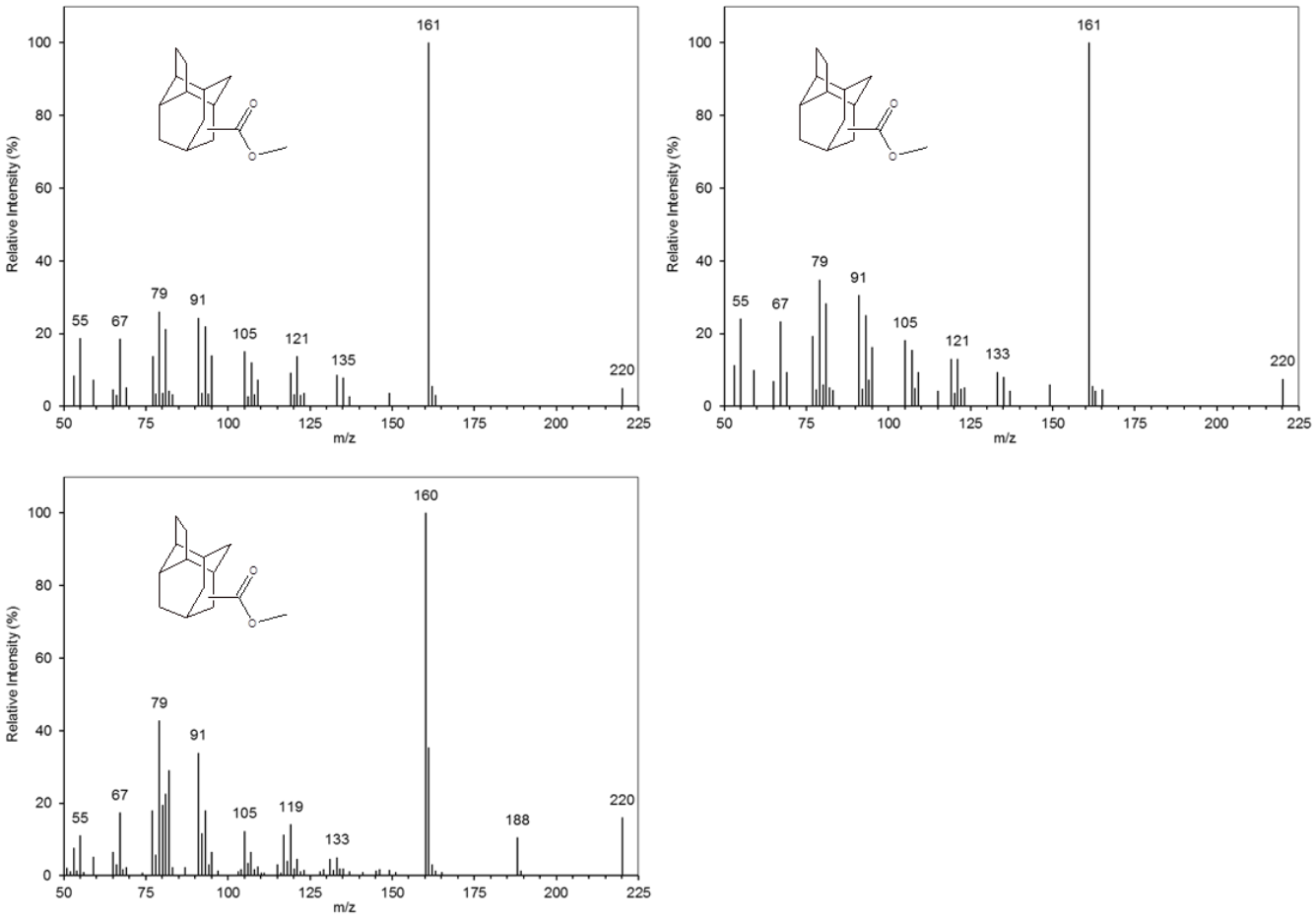

Figure S-9. Electron ionisation mass spectra of components in sample #2 NA methyl esters tentatively assigned as cyclopentano-adamantane acids after examination of the mass spectra of the corresponding hydrocarbons.

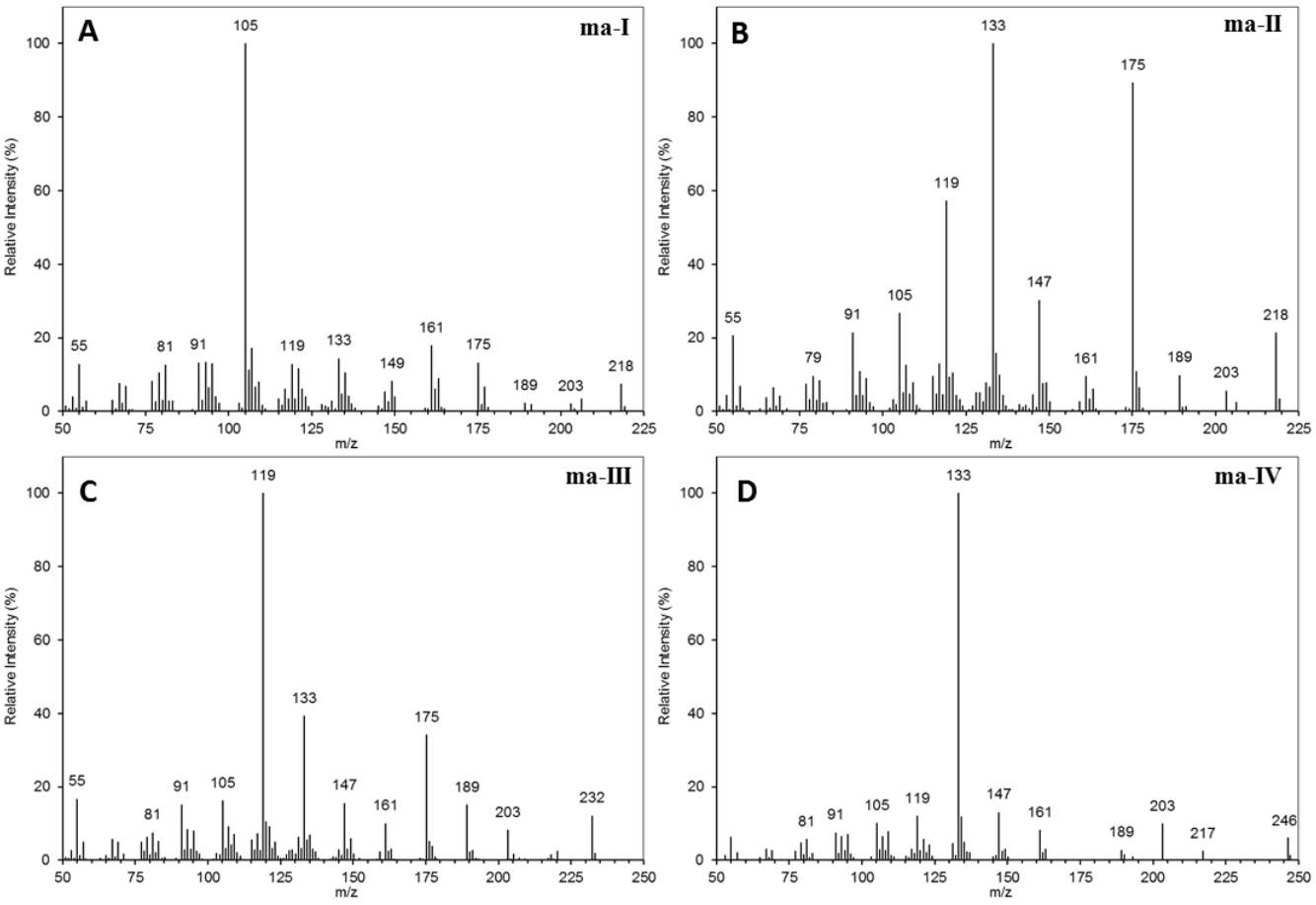

Figure S-10. (A-D) Example mass spectra of components assigned as C_{16-18} alkylbenzenes within the reduced aromatic fraction of sample #1.

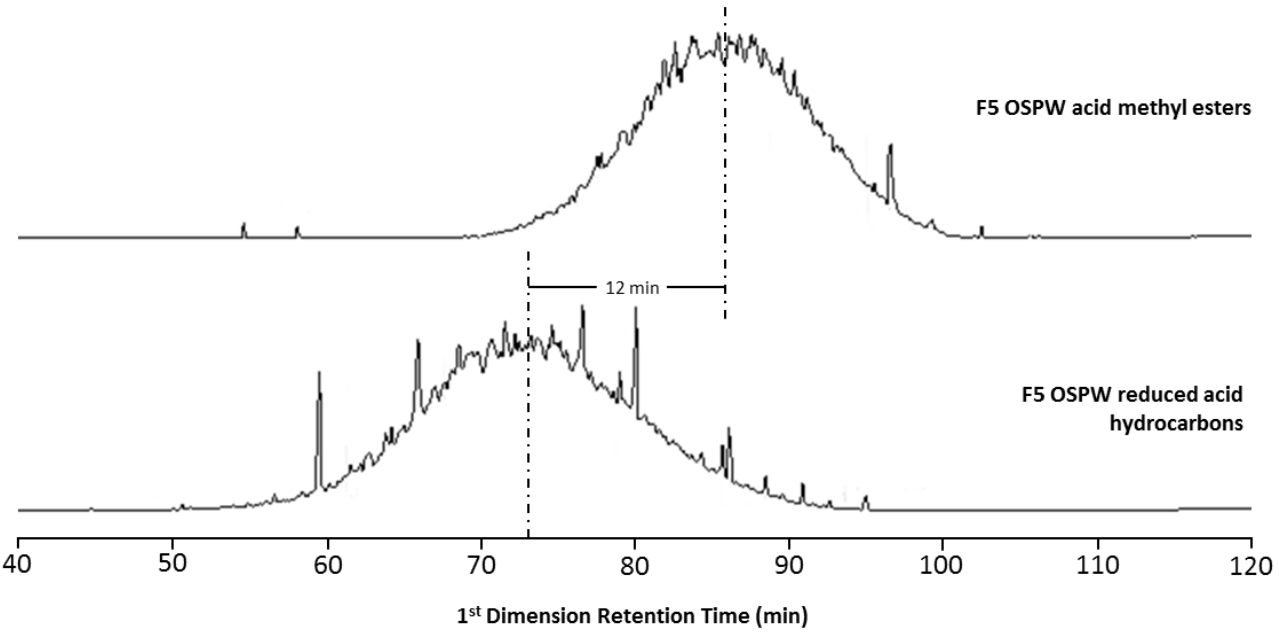

Figure S-11. Comparison of the GC-MS TIC chromatograms before (top) and after (bottom) reduction of the 'aromatic' fraction of sample #1 acid methyl esters, to the corresponding hydrocarbons.

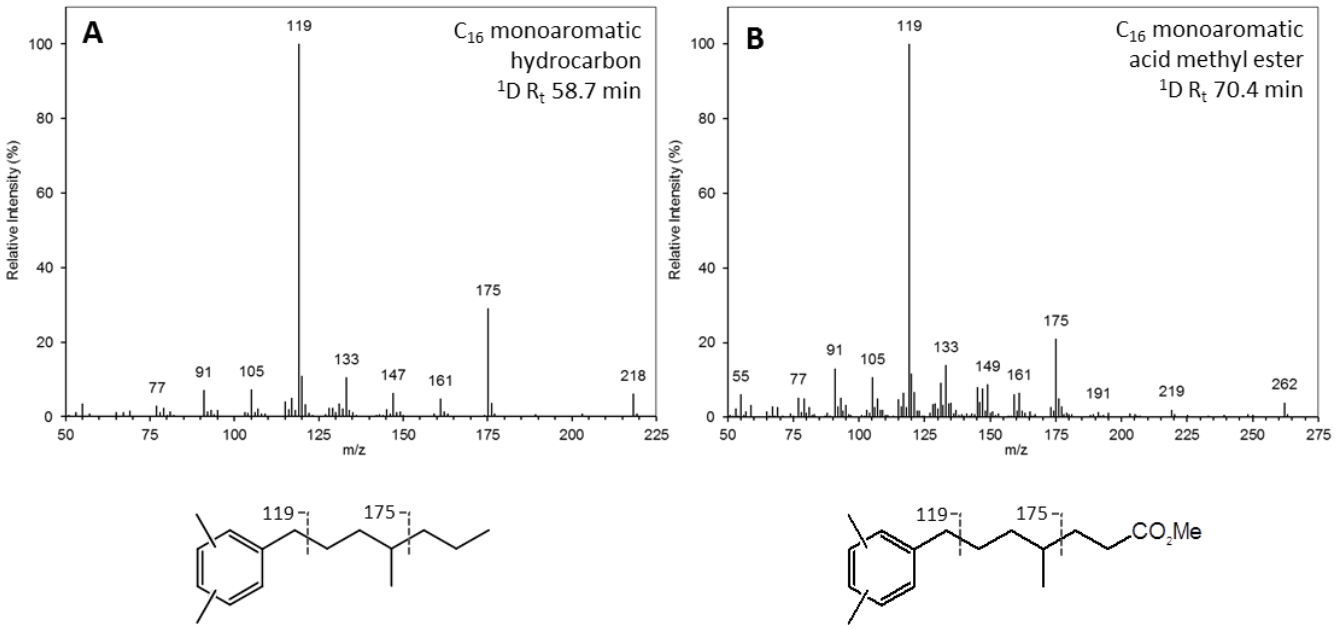

Figure S-12. (A) Mass spectrum of a C_{16} monoaromatic hydrocarbon in the reduced aromatic fraction of sample #1, assigned as a dimethyl branched alkylbenzene, based on comparison with reference spectra and mass spectral interpretation and (B) the mass spectrum of a C_{16} monoaromatic acid methyl ester, assigned as the corresponding alkylbenzene acid methyl ester before reduction of the aromatic fraction. Structures given are speculative, to demonstrate key fragment ions observed.

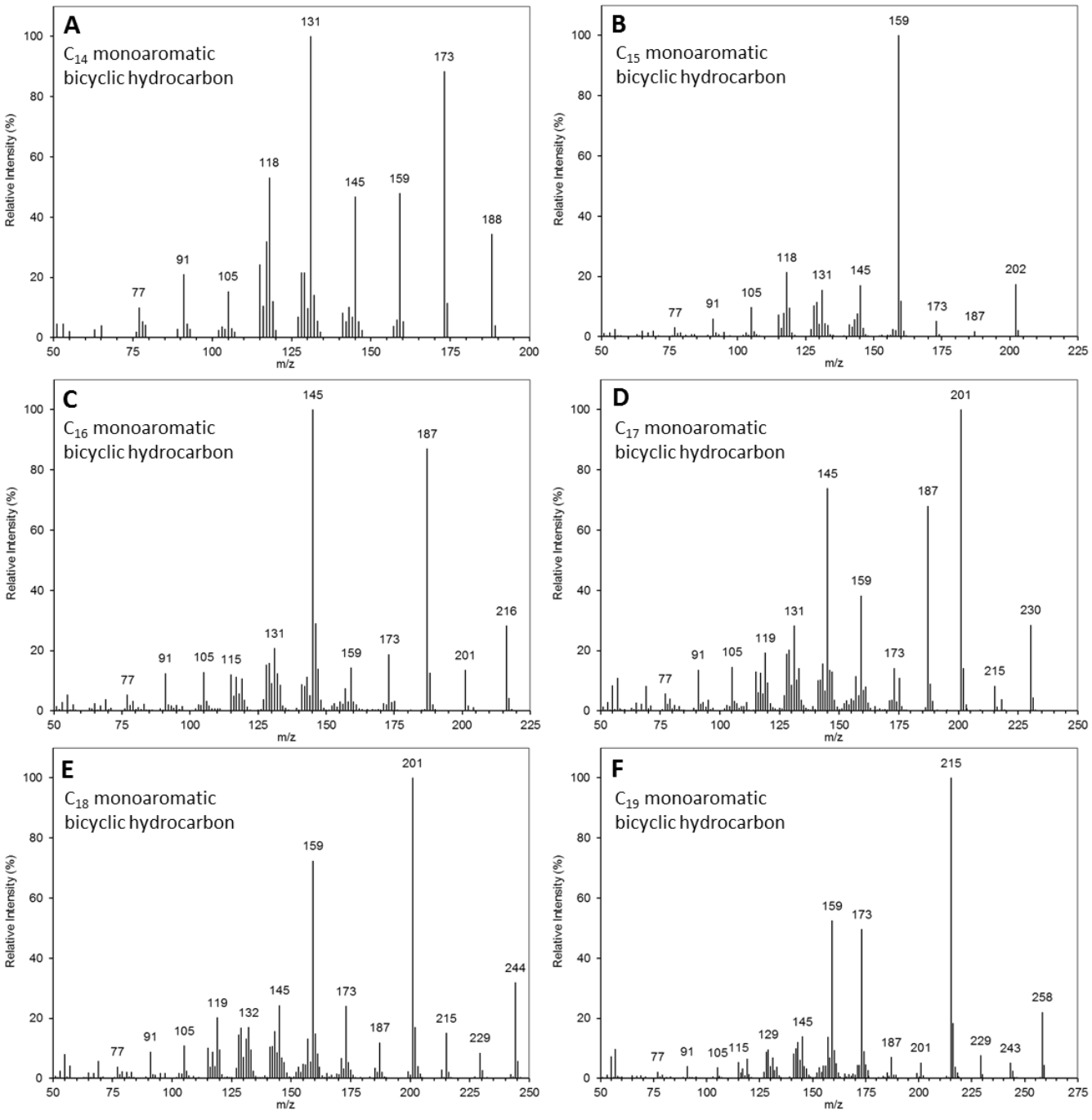

Figure S-13. (A-F) Mass spectra of C_{14-19} monoaromatic bicyclic hydrocarbons tentatively assigned based on mass spectral comparison and interpretation.

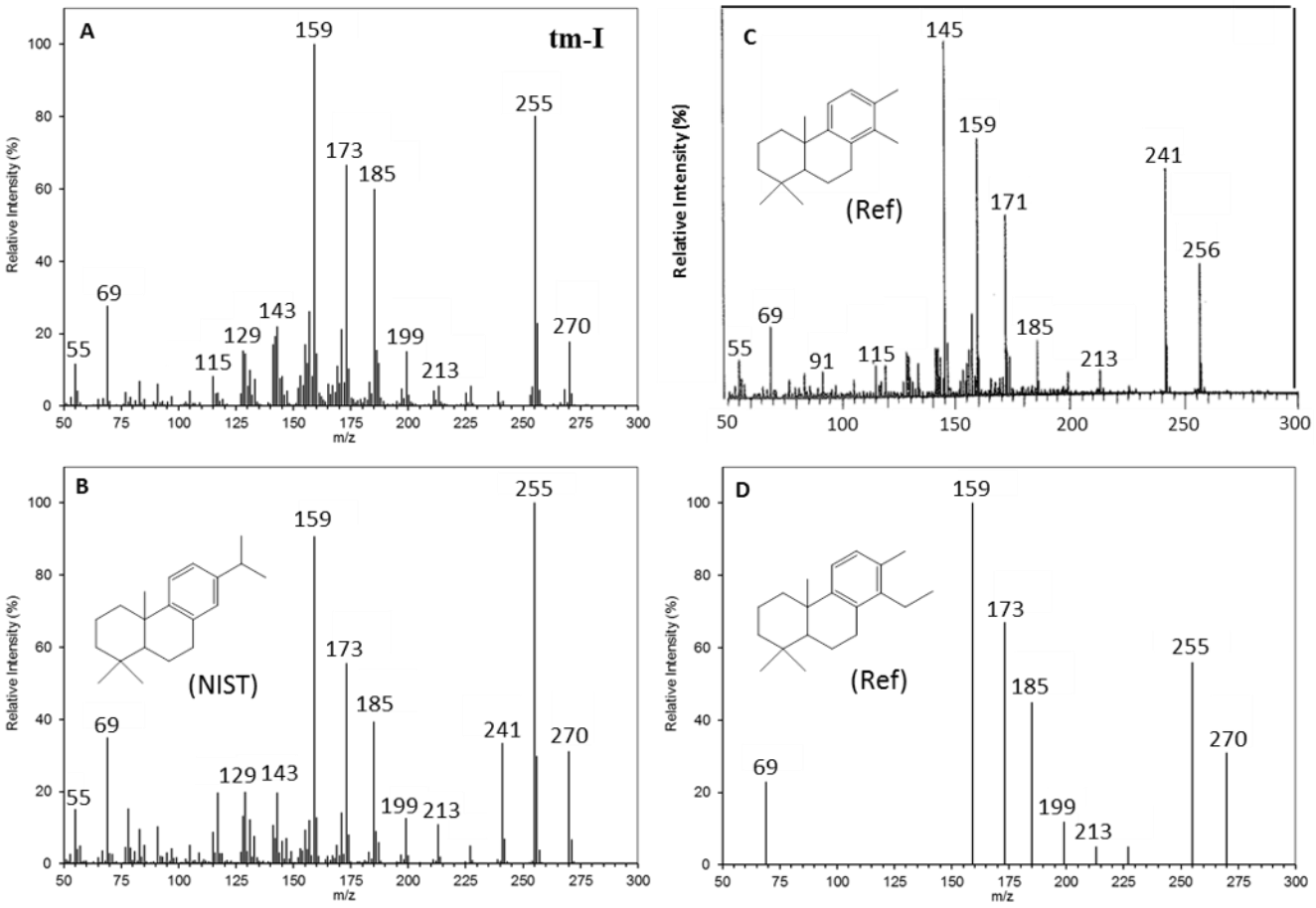

Figure S-14. (A) Mass spectrum of isomer tm-I, a C20 tricyclic monoaromatic hydrocarbon assigned as 13-methyl-14-ethylpodocarpa-8,11,13-triene after comparison with the mass spectra of (B) dehydroabietane plotted from the NIST MS Library, (C) 13,14-dimethylpodocarpa-8,11,13-triene reimaged from the spectrum reported byAzevedo et al. (1992) and (D) 13-methyl-14-ethylpodocarpa-8,11,13-triene replotted from the tabulated values reported by Azevedo et al. (1990).

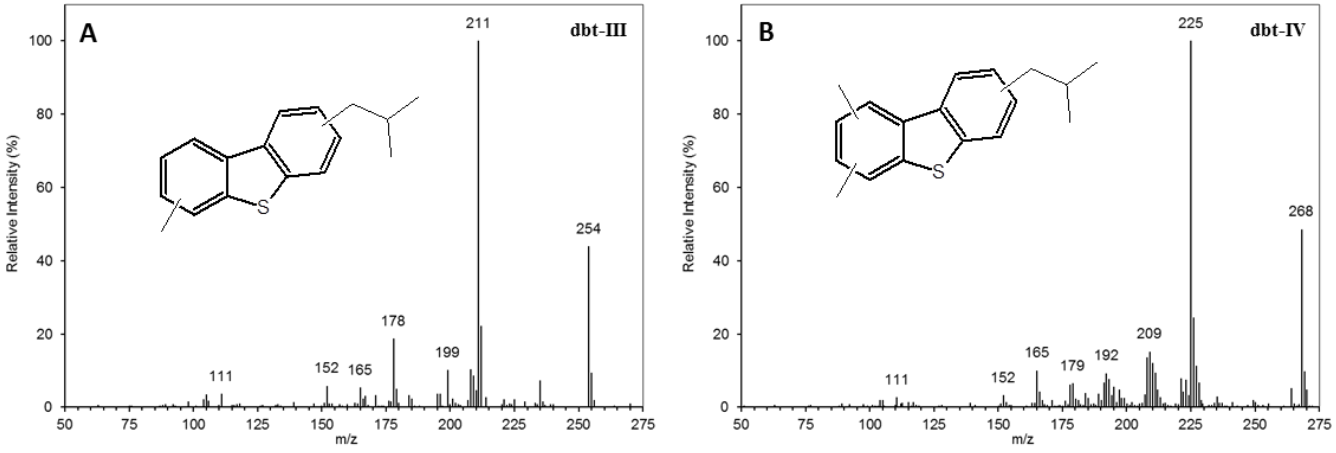

Figure S-15. (A and B) Assignment of dbt-III and -IV as methyl- and dimethyl- isobutyldibenzothiophenes.

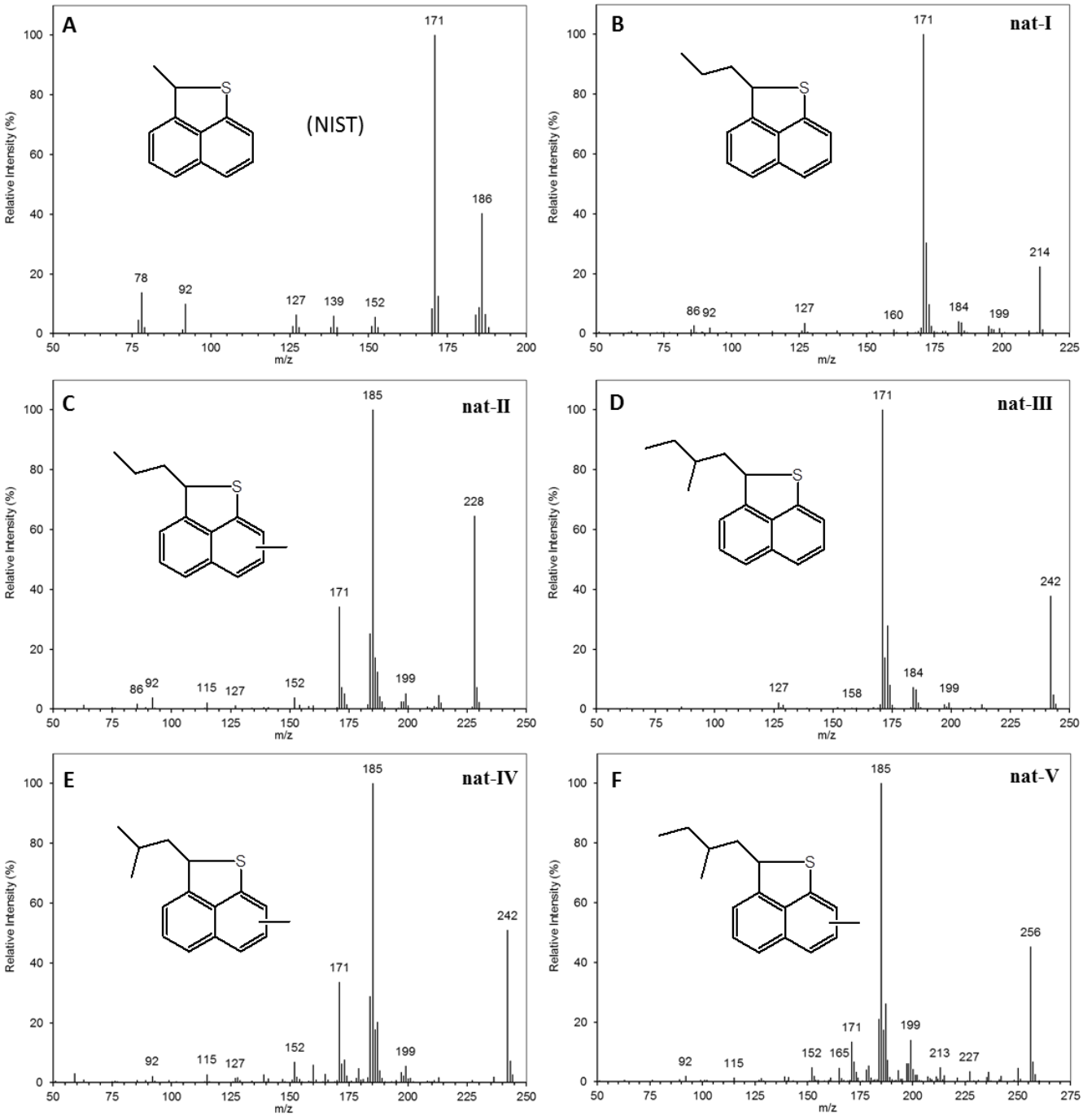

Figure S-16. Mass spectra of a series of isomers (nat-I to -V) in the reduced 'aromatic, sulphur' fraction of sample #1, tentatively assigned, after comparison with (A) the NIST spectrum of 2-methyl-2H-naphtho[1,8-bc]thiophene and their retention positions relative to authentic 4-propyl- and isobutyl- dibenzothiophene, as (B-F) 2-alkyl (C3-5) substituted 2H-naphtho[1,8-bc]thiophenes and methyl 2H-naphtho[1,8-bc]thiophenes.

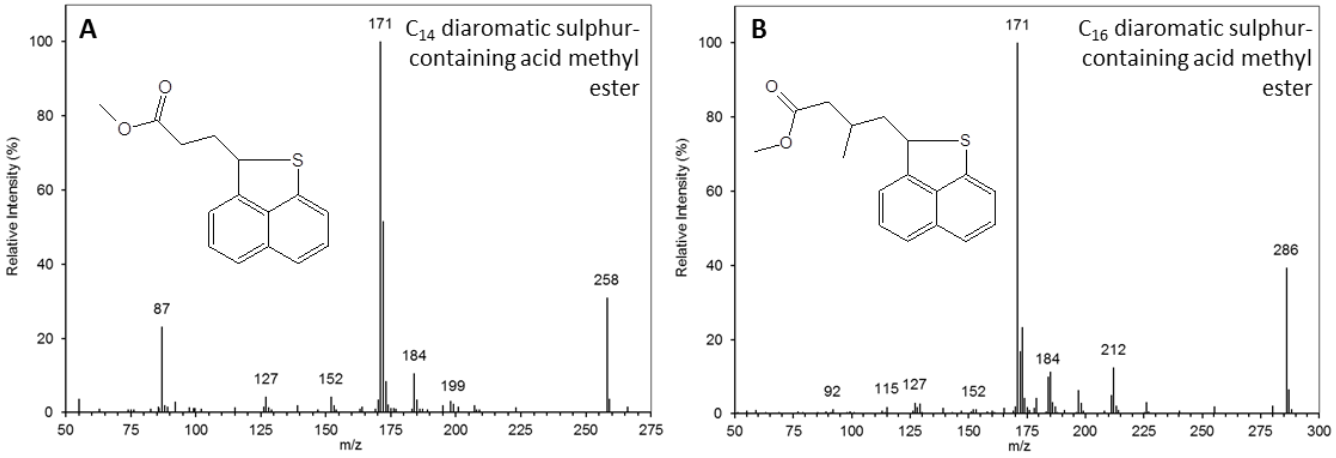

Figure S-17. (A and B) Example mass spectra of a C_{14} and C_{16} acid methyl ester in the 'aromatic, sulphur' fraction of sample #1, with molecular ions and retention positions consistent with condensed tricyclic, diaromatic sulphur-containing acid methyl esters, tentatively assigned as naphtho[1,8-bc]thiophene acids.

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